SEP 10 1948
©CIB 153208

VOLUME 6 NUMBER 9

NUTRITION REVIEWS

SEP 15 1948

September 1948

Nutrition in Diabetes 257
Lactate-Pyruvate Relations in the Blood of Man 259
Bone Rarefaction, Hypoprothrombinemia, and Hypervitaminosis A .. 261
Transfer of Selenium to Milk Proteins 263
Vitamin Requirements of the Horse 264
Action of Insulin on Ascorbic Acid Metabolism 268
Minimum Protein Requirements of Adult Dogs 269
Malignant Malnutrition in South Africa 270
Availability of Pantothenyl Alcohol to Human Beings 272
The Effect of DL-Methionine on the Healing of Wounds in Protein-Depleted Animals 274
Effect of Administered Iron During Pregnancy 275
Bacterial Utilization of Peptides 277
Diet and Tuberculosis Susceptibility in Mice 278
Hunger Edema in Children 279
Comparative Biologic Activity of Beta-Carotene and Vitamin A 281
Effect of Alloxan on Normal and Neoplastic Pancreatic Islet Cells in Man 282
The Structure and Activity of Pteroylglutamates 283
Dietary Impairment of Estrogen Response in the Immature Monkey .. 284
Effect of Thiamine Deficiency on Western Equine Encephalitis in Mice 285
Notes 287
Letter to the Editor 287
Sodium in Canned Foods 287
Composition of Sow's Milk 287
Colitis in the Folic Acid Deficient Monkey 288
Recent Nutrition Books 288

Published Monthly by

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK, N. Y.

COPYRIGHT, 1948, NUTRITION FOUNDATION INC., NEW YORK, N. Y., U. S. A

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 695 Huntington Ave., Boston, Mass.

Assistant Editor

IRENE M. KINSEY, Harvard University, School of Public Health, 695 Huntington Ave., Boston

Associate Editors

HUGH R. BUTT, M.D., Mayo Clinic, Rochester, Minn.
VERNON H. CHELDELIN, PH.D., Department of Chemistry, Oregon State College, Corvallis
WILLIAM J. DARBY, M.D., PH.D., School of Medicine, Vanderbilt University, Nashville
CHARLES S. DAVIDSON, M.D., Thorndike Memorial Laboratory, Boston City Hospital
WENDELL H. GRIFFITH, PH.D., Department of Biological Chemistry, School of Medicine, St. Louis University, St. Louis
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston
ESMOND E. SNELL, PH.D., Department of Biochemistry, University of Wisconsin, Madison
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester
M. M. WINTROBE, M.D., PH.D., School of Medicine, University of Utah, Salt Lake City

Editorial Committee

REGINALD M. ATWATER, M.D., American Public Health Assn.
GEORGE R. COWGILL, PH.D., Yale University
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
J. MURRAY LUCK, PH.D., Stanford University
JAMES S. MCLESTER, M.D., University of Alabama
HENRY C. SHERMAN, PH.D., Columbia University
HAZEL STIEBELING, PH.D., Bureau of Human Nutrition & Home Economics
LEROY VORIS, PH.D., National Research Council
RUSSELL M. WILDER, M.D., University of Minnesota
JAMES R. WILSON, M.D., American Medical Association
JOHN B. YOUMANS, M.D., University of Illinois
H. M. SINCLAIR, M.D., Oxford University
CORRESPONDENT FOR ENGLAND
FREDERICK F. TISDALL, M.D., University of Toronto
CORRESPONDENT FOR CANADA

Subscriptions should be sent to the Nutrition Foundation, Inc., Chrysler Building, New York, N. Y. Rate $2.00 per year in the United States and Canada; $2.50 in other countries.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved. NUTRICION, a Spanish edition of NUTRITION REVIEWS is published by La Prensa Medica Mexicana, Zacatecas 21, Mexico, D.F. Subscriptions should be sent to this address. Rate $2.00 (U.S. currency) or $10.00 (pesos mexicanos).

NUTRITION REVIEWS

VOL. 6 SEPTEMBER, 1948 NO. 9

NUTRITION IN DIABETES

The aim of nutrition in diabetes should be twofold: first, to control and ameliorate the disease; and secondly, to prevent or delay as far as present knowledge permits, the complications of the disease. Regarding the first aim, it has long been known that tolerance to glucose could be increased in diabetic patients simply by adequate control of carbohydrate metabolism with insulin and a proper diet. Opinions have varied as to what constitutes a proper diet, but most observers are now agreed that it should vary little from the dietary standard of normal people. There is a tendency at present to prescribe a somewhat lower carbohydrate and total caloric intake than normal to avoid obesity, reduce insulin requirement, and facilitate regulation; however, the greater caloric needs of growing children and active adults are being recognized.

H. P. Himsworth (*Lancet* **II,** *171 (1939)*) re-emphasized that sensitivity to insulin varies directly with the amount of carbohydrate in the diet. As he expresses it, the greater the deficiency in carbohydrate, the less is the response to a given dose of insulin and the greater the "head of pressure," i.e., the blood sugar level, necessary to bring about the oxidation of a given amount of glucose. It is apparent, therefore, that in the diabetic patient as in the normal person, increasing the intake of carbohydrate will, within reasonable limits, increase the efficiency of action, not only of exogenous but also of endogenous insulin. Thus a diabetic patient may be better controlled with less insulin by a judicious increase in his carbohydrate intake.

From the nutritional standpoint it is helpful to divide diabetic patients into two broad groups, insulin sensitive and insulin insensitive. The insulin sensitive group comprises in the main the younger patients who are more apt to be at or below ideal weight and who have a rapid fall in blood sugar levels following the injection of insulin. It is this group in particular which shows an increased sensitivity to insulin with an increased carbohydrate intake.

The insulin insensitive group is composed chiefly of patients whose diabetes began in later life. They are more likely to be overweight and they show a slower, less marked but more prolonged fall in blood sugar levels following the same dose of insulin than do the insulin sensitive patients. Moreover, they show little or no tendency to increase their sensitivity to insulin with an increase in carbohydrate intake.

The different dietary requirements of these two groups are obvious. The insulin sensitive group should be maintained on a relatively high carbohydrate intake. In this way insulin action will be most efficient and the likelihood of acidosis diminished. Himsworth goes so far as to say that conceivably diabetes might be produced in an individual hereditarily predisposed to the disease by too great a restriction of carbohydrate. This view is based on the theory that carbohydrate deficiency stimulates the pituitary to secrete its diabetogenic and ketogenic hormones in order to facilitate the production of energy from protein and fat. These hormones in turn may, as shown by F. G. Young (*Lancet* **II,** *372 (1937)*), actually cause destruction of the Islands of Langerhans, if they act in sufficient quantity for a long period of time.

On the other hand, there is a recent note of warning against overfeeding with carbohydrate. F. C. Dohan and F. D. W. Lukens (*Federation Proceedings* **6,** *97 (1947)*) have produced permanent diabetes in cats merely by the prolonged administration of excessive

quantities of glucose. This achievement has led to the theory of islet exhaustion due to oversecretion of insulin, and conversely, to the hypothesis that the pancreas may be put at rest and given an opportunity to regenerate if sufficient exogenous insulin is supplied to provide for complete oxidation of dietary carbohydrate. It may be concluded, therefore, that there is danger both in overfeeding and underfeeding the insulin sensitive diabetic, and that the best diet is that which is closest to the normal for any given individual's ideal weight.

The dietary management of the insulin insensitive diabetic is usually more directly concerned with reduction of obesity. It is this group in particular who should receive the lower carbohydrate and caloric intake. Nevertheless, it is important here, too, that sufficient carbohydrate be prescribed to prevent the necessity for burning fat and protein with its consequent stimulation of the pituitary diabetogenic principle. Only when the need for weight reduction is imperative should an individual be allowed to burn his own excess fat.

The principal complications of diabetes with which the nutritionist is concerned are the vascular changes and the neuropathies. The first and most important requisite for diminishing the threat of early degenerative vascular disease is adequate control of carbohydrate metabolism to prevent the hyperlipemia and hypercholesterolemia which appear whenever fat is called upon for oxidation. Dr. E. P. Joslin and his group (Editorial, *J. Am. Med. Assn.* **135,** *1074 (1947)*) have shown clearly the direct relation between the severity of arteriosclerosis and the number of episodes of acidosis in diabetic individuals. It is just as important to remember, however, that the mild diabetic who is inadequately controlled for long periods of time, although never in clinical acidosis, is even more prone to develop arteriosclerosis because there is no obvious necessity for better control, as there is in the severe diabetic who must be continuously treated to prevent coma and death.

It is pertinent to inquire what if any specific nutritional measures might be employed to combat arteriosclerosis. We know that an important feature of this disease is the abnormal and excessive deposition of cholesterol and calcium in the arterial wall. Presumably the level of cholesterol in the blood reflects to some extent the likelihood of its deposition in the tissues. In addition to minimizing fat mobilization by adequate control of carbohydrate metabolism, there are other methods of lowering serum and tissue cholesterol levels which deserve further study. For instance, it has long been known that iodine will prevent arteriosclerosis in rabbits fed cholesterol in excessive amounts (I. H. Page and W. G. Bernhard, *Arch. Path.* **19,** *530 (1935)*). Also, both the eggplant and the Jerusalem artichoke possess the capacity to lower the level of cholesterol in the serum, presumably by increasing its excretion in the bile (A. H. Roffo, *Yale J. Biol. Med.* **18,** *25 (1945)*).

The existence of a hitherto unrecognized nutritional factor controlling the extraskeletal deposition of calcium has been demonstrated by R. Wulzen and A. M. Bahrs (*Am. J. Physiol.* **133,** *P500 (1941)*). A deficiency of this so-called "antistiffness" or Wulzen factor in the guinea pig leads first to stiffness of the joints and later to deposition of calcium in muscle, aorta, and subcutaneous tissues. Raw cane juice has been shown to be particularly rich in this factor and more recently a pure sterol, ergostanol, has been found to exert the same action (J. J. Oleson *et al.*, *J. Biol. Chem.* **171** *1 (1947)*). This discovery opens up a wide field of exploration relating to abnormal calcification and the degenerative diseases.

Another point of nutritional importance is the correlation between hypoalbuminemia and diabetic retinitis. This is of particular interest in that improvement in the retinitis has been reported following administration of high protein diets (R. W. Schneider, L. A.

Lewis, and E. P. McCullagh, *Am. J. Med. Sci.* **212,** *462 (1946)*). Thus, it may well be advisable to revise upwards our estimates of the protein requirements of diabetics. Another argument in favor of high protein diets is the frequency of liver enlargement in diabetes due either to fatty infiltration or hydropic degeneration (N. B. Krarup and P. Iversen, *Acta med. Scandinav.* **105,** *433 (1940)*). As in other forms of liver disease, a high protein intake has been the most valuable form of treatment thus far found. With restoration of normal liver function, insulin action will be more efficient and diabetes better controlled.

Animal protein is also one of the best dietary sources of potassium and phosphorus upon which the body is dependent for the normal oxidation of carbohydrate. This dependency becomes particularly apparent during treatment of diabetic coma with large amounts of glucose and insulin. In such situations the demand for phosphorus and potassium may exceed the supply and near deaths have been reported from respiratory paralysis associated with a low serum potassium level (J. W. Holler, *J. Am. Med. Assn.* **131,** *1186 (1946)*).

With the increased knowledge of the function of vitamins in carbohydrate metabolism and their widespread, if sometimes indiscriminate, use in general medical practice, the problem of primary diabetic neuritis has become less acute. Nevertheless, it is an ever present threat to the diabetic patient and of particular importance to the nutritionist because it is one of the complications of diabetes which can be prevented, or cured, provided it is treated sufficiently early. It is merely necessary to remember that at least three of the "B-vitamins" are as essential as insulin in the chain of glucose oxidation, and that nerve tissue depends principally on the metabolism of carbohydrate for its function. The vitamin need of diabetic patients appears to vary directly with their insulin requirement, or, more properly with their rate of glucose utilization. Those receiving inadequate amounts of insulin may get along on submaintenance levels of vitamin intake, only to have their vitamin deficiency become manifest when carbohydrate oxidation is accelerated by increasing their insulin dosage. Essentially, therefore, the prevention of diabetic neuritis consists in maintaining the patient on adequate and constant amounts of insulin and assuring him an excess intake of the "B-vitamins" to take care of fluctuations in the rate of carbohydrate utilization which may occur as a result of variations in insulin sensitivity. The treatment of an established neuritis involves the use of large but balanced quantities of the water soluble vitamins administered for long periods of time. With patience and persistence gratifying results may be obtained.

KENDALL EMERSON, JR.
Harvard Medical School and
Peter Bent Brigham Hospital
Boston

LACTATE-PYRUVATE RELATIONS IN THE BLOOD OF MAN

An increased amount of pyruvic acid occurs in the blood and tissues of animals which are deficient in thiamine and in human beings who are suffering from beriberi (B. S. Platt and G. D. Lu, *Quart. J. Med.* **5,** *355 (1936)*). In acute deficiency of thiamine in man the pyruvic acid contained in the blood during fasting tends to be higher than normal; even in men on a restricted intake of the "B vitamins" the relation of lactic to pyruvic acid in the blood is altered (*Nutrition Reviews* **1,** *24 (1942)*, A. Keys *et al.*, *Am. J. Physiol.* **144,** *5 (1945)*). Likewise, an increase of pyruvic acid in the blood has been noted in cases of addiction to alcohol with associated acute peripheral neu-

ropathy, in Wernicke's syndrome, in patients with high fever (*Nutrition Reviews* **1,** *148 (1943)*), and in patients with hepatic, thyroid, renal, and neoplastic diseases (*Ibid.* **2,** *305 (1944)*). Elevations in the amount of pyruvic acid also have been noted in rats when shock was induced by bleeding.

The difficulty in experimental studies of pyruvate metabolism is that so many factors are known to influence the concentrations of pyruvic acid in the blood (*Nutrition Reviews* **5,** *241 (1947)*). It has been reported that biotin in some form acts as a catalyst in the formation of oxalacetate from pyruvate and carbon dioxide (*Ibid.* **5,** *346 (1947)*). The possible role of pantothenic acid in the oxidation of pyruvic acid also has been considered (*Ibid.* **5,** *331 (1947)*).

G. A. Goldsmith (*Am. J. Med. Sci.* **215,** *182 (1948)*) undertook a further study of the effect of certain physiologic changes on the concentration and relation of lactic and pyruvic acid in the blood of normal persons and of patients with various pathologic states. The hope was that such a study might produce methods which would assist in the diagnosis of thiamine deficiency in man. The subjects were 29 presumably normal persons and 41 patients who were being treated for a variety of diseases in the hospitals. Concentrations of lactic and pyruvic acids in the blood were determined during fasting, after meals, after administration of glucose, during rest, after light and strenuous exercise, and in a few instances, after electric shock therapy. Determinations of pyruvic acid were made according to the method of E. Bueding and H. Wortis (*J. Biol. Chem.* **133,** *585 (1940)*), and of lactic acid by the method of S. B. Barker and W. H. Summerson (*Ibid.* **138,** *535 (1941)*).

In normal persons, the concentration of pyruvic and lactic acid in the blood and the lactate-pyruvate ratios were determined under conditions of rest, exercise, and alimentation. In 21 persons the mean finding in the basal state for pyruvic acid was 1 mg. per 100 ml.; lactic acid was 9.3 mg. per 100 ml.; therefore the lactate-pyruvate ratio was 1:9.3 with a standard deviation of ± 1.7. After light exercise in the fasting state the findings were similar to those obtained while the patient was at rest. Light exercise taken two or three hours after breakfast was followed by an increase in blood lactic acid, while pyruvic acid remained unchanged. Administration of glucose was followed by an increase in both pyruvic and lactic acids in 9 of 10 subjects. The maximal rise occurred after an interval of thirty to sixty minutes in 6 instances and after ninety to one hundred and twenty minutes in 3 instances. At the end of two or three hours the concentrations of these metabolites returned to fasting levels. In all instances, brief periods of strenuous exercise were followed by moderate increase in pyruvic acid but by marked increase in lactic acid. The greatest increases in lactate and pyruvate occurred five to fifteen minutes after exercise; even at the end of thirty minutes these values were comparatively high. Electric shock therapy, in which convulsive seizures were produced, was followed by markedly increased blood concentrations of both lactic and pyruvic acids, but the mean lactate-pyruvate ratio was essentially the same as that noted after strenuous exercise. Undoubtedly, anoxia and muscular activity are the factors responsible for the rise in the concentrations of lactic and pyruvic acids which follows electric shock therapy. In those few patients in whom electric shock therapy failed to produce a seizure, the rise in both lactic and pyruvic acid was less marked than when muscular contractions occurred.

In 41 patients suffering from various diseases it was noted that, in those with a deficiency of thiamine, riboflavin, or niacin, the mean pyruvic acid was elevated above the normal and there was a decrease in the mean lactate-pyruvate ratio. In patients with cardiac disease the mean pyruvic acid was slightly increased and the mean lactic acid decreased with a resultant lowering of the

ratio of these metabolites. Although 19 of these 41 patients with miscellaneous conditions were afebrile the mean lactate-pyruvate ratio was essentially normal.

Six of the 41 patients had clinical evidence of deficiency of thiamine and 3 of these 6 had an increase in pyruvic acid above 1.3 mg. In all 6 patients the lactate-pyruvate ratio was somewhat low and in 3 instances it was less than 1:7. One of these 6 patients had been delivered of a child who had a condition which was diagnosed as congenital beriberi. She was retested after thiamine had been administered for fourteen days and clinical improvement had occurred; there was a marked change in the biochemical findings. The pyruvic acid level had decreased from 1.16 mg. per 100 ml. to 0.52 mg., and the lactate-pyruvate ratio had increased from 1:6.9 to 1:10.1.

Nine patients with heart disease were studied. Pyruvic acid was elevated in 3 subjects, while the lactate-pyruvate ratio was below 1:7 in 4 subjects. Glucose was given to 4 patients and the data noted corroborated the findings obtained during fasting. The abnormalities of pyruvate metabolism did not appear to be related to the presence of heart failure or to its severity. Two of the individuals who had heart disease in whom the lactate-pyruvate ratio was low were treated with thiamine and retested after two weeks. After treatment there was a decrease in the ratio and a decrease in the already excessive amount of pyruvate. These data indicate that the low lactate-pyruvate ratio found in 4 of the 9 patients with heart disease apparently was due to deficiency in thiamine. These deficiencies may have been of dietary origin or due to a relative deficiency resulting from metabolic changes of anoxia associated with heart disease. In the presence of anoxia, lactic acid usually is increased to a greater extent than is pyruvic acid, but other enzyme systems have also been shown to be decreased in anoxic states. In experimental shock in anoxia the administration of thiamine likewise leads to a reduction in pyruvic acid (M. E. Greig and W. M. Govier, *J. Pharmacol. Exp. Therap.* **79,** *169 (1943)*).

Since the concentration of pyruvic acid in the blood depends greatly on the degree of physical activity the interpretation of slight changes in this constituent is difficult. Certainly, from this study it would appear that well controlled determinations of the relation of lactic and pyruvic acids in the blood might be of some assistance in evaluating the deficiency of thiamine in certain pathologic states. However, despite the great desirability of having an objective metabolic test for the sufficiency of thiamine nutrition, it cannot yet be claimed that the lactate-pyruvate determination reveals any lesser degree of deficiency than determination of thiamine itself in body fluids.

BONE RAREFACTION, HYPOPROTHROMBINEMIA, AND HYPERVITAMINOSIS A

Although vitamin A is relatively nontoxic, administration of greatly excessive amounts is injurious. Skeletal abnormalities, hemorrhage, and anemia have been observed in a child who had received for several years 240,000 I.U. of vitamin A daily in the form of halibut liver oil (*Nutrition Reviews* **2,** *148 (1944)*). It appears that most, if not all, of the toxicity of the liver of the polar bear may be due to its high concentration of the vitamin (*Ibid.* **2,** *63 (1944)*). Among the commonly observed effects of hypervitaminosis A in rats are roughening of the skin, alopecia, retarded growth, rarefaction of bones with spontaneous fractures, and subcutaneous and intramuscular hemorrhage (*Ibid.* **5,** *49 (1947)*).

An effect of vitamin A on bones is well established and it is generally agreed that this vitamin is essential for normal skeletal

development. Observed lesions of the central nervous system in vitamin A deficiency are probably related to skeletal abnormalities (*Nutrition Reviews* **1,** *419* (*1943*)). The extraordinary weakening of bones in rats fed large amounts of vitamin A is illustrated by the results of studies of certain so-called "telang" livers obtained from cattle condemned at the slaughter house because of telangiectasis. Growth failure, paralysis, and multiple fractures were observed in young rats restricted to a diet of telang liver supplemented with calcium carbonate (P. L. Pavcek, E. J. Herbst, and C. A. Elvehjem, *J. Nutrition* **30,** *1* (*1945*)). Normal and telang livers were similar in their content of carotene, ascorbic acid, choline, pantothenic acid, niacin, biotin, fat, and copper. Telang livers contained 50 per cent more iron, and 18 of 26 showed preformed vitamin A levels of 10 to 60 mg. per cent. Most of the normal livers contained less than 10 mg. per cent of true vitamin A. Fractures occurred within one week in young rats fed those telang livers which supplied 20,000 or more I.U. of vitamin A daily. Similar pathology resulted when a diet of nontoxic telang liver was supplemented with sufficient halibut liver oil or crystalline vitamin A to provide a daily intake of 15,000 to 20,000 I.U.

The hemorrhagic condition in hypervitaminosis A in rats is associated with a prolonged clotting time which responds to administration of vitamin K. S. E. Walker, E. Eylenburg, and T. Moore (*Biochem J.* **41,** *575* (*1947*)) have confirmed this significant observation of R. F. Light, R. P. Alscher, and C. N. Frey (*Science* **100,** *225* (*1944*)). Light *et al.* noted delayed coagulation of the blood of rats receiving 15,000 I.U. of vitamin A daily but not following the administration of 8000 I.U. Fatal cerebral hemorrhage was common in rats given 35,000 I.U. The alcohol and ester forms of vitamin A produced similar hypoprothrombinemias, but carotene was without effect. A daily dose of 25 micrograms of vitamin K maintained a normal prothrombin level, 10 micrograms giving incomplete protection. There was no indication in these experiments that other manifestations of hypervitaminosis A were influenced by vitamin K.

Walker *et al.* reported similar observations on the protective action of vitamin K. In their experiments hemorrhage was variable in intensity and in distribution. It was more evident in older rats, whereas spontaneous fractures were more common in younger animals. Vitamin K prevented hemorrhage consistently in animals weighing 85 g. or more. In smaller animals hemorrhage sometimes occurred despite vitamin K therapy. Daily administration of 50 mg. of ascorbic acid was without effect on the hypoprothrombinemia of the hypervitaminotic rats. Of interest is the observation that the blood of rats receiving toxic amounts of vitamin A appeared thin and watery. Determination of the ratio of plasma to cell volume gave values of 2.2 for the experimental rats, 1.2 for the controls, and 1.6 for the group treated with vitamin K. No correlation between the occurrence of fractures and hemorrhage was found in these experiments. The probability that the two conditions represented unrelated effects of vitamin A toxicity was supported by the failure to find evidence of increased fragility of bone in rats with hypoprothrombinemia induced by the administration of Dicumarol.

It has been assumed in this review that the reported signs of toxicity were, in fact, evidence of hypervitaminosis A and were not the result of an unknown toxic substance in the vitamin A preparations that were used. The latter possibility has not been definitely ruled out. E. B. Vedder and C. Rosenberg (*J. Nutrition* **16,** *57* (*1938*)) have found little correlation between the toxicity and vitamin A content of jewfish liver oils. Rarefaction of bones and hemorrhage were observed, but only when 100,000 or more units of vitamin A were administered daily. Furthermore, injury to the rats was prevented by ascorbic acid which Walker *et al.* found wholly ineffective. Repeated observations on syn-

thetic, highly purified samples of vitamin A will be necessary before it can be stated with certainty that the vitamin itself produces the described effects. The conflicting reports suggest that variables in experimental procedures influence the onset and severity of the toxic manifestations, some of which may be indirect rather than direct results. These studies are important because they indicate valuable therapeutic measures in cases of accidental ingestion of excessive quantities of certain potent preparations of vitamin A and because they furnish pertinent leads for further investigations.

TRANSFER OF SELENIUM TO MILK PROTEINS

A. L. Moxon (*South Dakota Agr. Exp. Sta. Bull.* **311** (*1937*)) observed that selenium, the element which is the cause of "alkali disease" in cattle, was transferred through the milk of cows grazing in seleniferous areas in sufficient concentration to produce all the prominent signs of "alkali disease" in nursing calves. M. I. Smith, B. B. Westfall, and E. F. Stohlman (*Pub. Health Reports* **52,** *1171* (*1937*)) reported that the selenium content of milk from cows in regions where the plants contained selenium varied from 17 to 127 micrograms per 100 ml.

K. P. McConnell (*J. Biol. Chem.* **173,** *653* (*1948*)) made investigations of the distribution of selenium in the milk of rats after subcutaneous injection of subtoxic amounts of sodium selenate containing radioactive selenium. Five lactating rats, each having 3 to 6 pups which were 2 or 3 days old, were injected with from one to four doses of the labeled sodium selenate. Twenty-four hours after the last parental injection, the pups were killed by decapitation and wet ashed individually. Radioactivity of the ash was determined in a Geiger-Müller dip counter. Amounts of sodium selenate equivalent to between 0.33 and 2.3 per cent of the total weight injected into the mother rat were recovered from each suckling pup. When expressed on the basis of the percentage of the sodium selenate which was detected per litter, between 2.0 and 9.3 per cent of the injected dose was found to be present. These values obviously represent only a portion of the selenium secreted by the mammary gland during the twenty-four hour test period; additional amounts received through the milk must have been excreted by the young rats through the kidney and gastrointestinal tract. The mammary gland of one of the adult rats was found to contain 0.058 per cent of the administered sodium selenate per gram of tissue twenty-four hours after the last injection. This amount corresponded roughly to the concentration of selenium in the tissues of the rat pups.

In a second experiment, 2 lactating rats with 6 and 9 pups, respectively, 10 to 14 days of age, were injected with radioactive selenium as sodium selenate. The pups were separated from the dams for twelve to eighteen hours after the selenate injection, and then permitted a limited one to three hour suckling period. The pups were then killed and the stomach contents, consisting of milk curd, removed. The milk curd of each litter was pooled and the two samples agitated with benzoic acid to break up the curd. Total proteins of the milk from one litter were precipitated with trichloracetic acid. The milk from the second litter was divided into three aliquots. The first was acidified to pH 4.6 to precipitate casein; the second was saturated with magnesium sulfate at 20°C. to precipitate casein and globulin; and the third was heated with 4 per cent trichloracetic acid to yield the total protein fraction. Radioactivity determinations were made on the precipitate and filtrate for each of the four samples after the precipitate had been washed twice. The milk from the first litter contained 0.515 per cent of the administered dose per milliliter,

while that from the second litter contained 0.913 per cent per milliliter. Approximately 54 per cent of the selenium in the milk from the second litter were present in the washed casein precipitate, while 40 and 7 per cent were present in the albumin and globulin fractions, respectively. Thus, the selenium which had been administered to the rats in inorganic form had been incorporated into organic combination in the three milk proteins, and no appreciable amount was present in inorganic form in the aqueous phase of the milk. The amount of selenium present in the various protein fractions appeared to be roughly proportional to the size of the fraction.

Because of the difficulties inherent in studying the distribution of selenium in various tissue fractions prior to the use of the radioactive isotope, there is little knowledge of the metabolism of selenium. In like fashion, the pathways through which organic and inorganic forms of the closely allied element, sulfur, are metabolized have been relatively unknown until recently. D. D. Dziewiatkowski (*J. Biol. Chem.* **164,** *165 (1946)*) demonstrated that sulfide sulfur containing radioactive sulfur was utilized by the rat for the synthesis of cystine, and C. V. Smythe and D. Halliday (*Ibid.* **144,** *237 (1942)*) demonstrated in *in vitro* studies that inorganic radioactive sulfur was converted in the presence of a suitable liver enryme preparation into organic sulfur. The metabolism of inorganic selenium to an organic selenium protein complex may have proceeded through similar reactions to be incorporated into similar amino acids possibly even on a competitive or replacement basis with sulfur.

This is not the first evidence that inorganic selenium is incorporated into protein complexes in animal tissues. B. B. Westfall and M. I. Smith (*Pub. Health Reports* **55,** *1575 (1940)*) demonstrated that administration of inorganic selenium to experimental animals resulted in accumulation of selenium in the proteins of the liver and into the plasma fractions, while McConnell (*loc. cit.*) has shown that selenium is transferred into proteins of the liver, into muscle, into the plasma fraction, and into hemoglobin. The rapidity with which selenium becomes incorporated into the integral units of the body and the extensiveness of this phenomenon may be one of the factors responsible for the extreme toxicity of selenium in the animal body.

VITAMIN REQUIREMENTS OF THE HORSE

During the past decade attention has been focused upon obtaining optimum nutrition for farm animals. Poultry and dairy cattle have been studied intensively. By contrast, studies of the nutrition of the horse are time-consuming and expensive, and the economic importance of the horse is declining steadily, although breeding for such special purposes as racing and cavalry continues. The latter group has furnished a large part of the available information on the nutrition of the horse.

Vitamin A deficiency in the horse was noted by D. Meadows (*Vet. J.* **26,** *140 (Oct. 1919)*) who observed night blindness in horses and camels in Persia, but was unaware of its cause. Later, V. Klemola (*Biedermann's Zentr. B. Tiernähr.* **5,** *657 (1933)*) noted marked changes in the hoofs of horses in the Finnish army. Ten to 15 per cent of the cavalry horses and 20 to 30 per cent of the artillery horses suffered brittleness, abnormal horn growth with furrows and cracks, and a spongy crust on the surface of the hoof wall toward the heels. The diet at that time consisted of oats, dry hay, and oat straw; no green fodder, and only little pasture were available. Analysis of samples of the hay showed the absence of vitamin A. The condition was improved considerably

in 113 horses by feeding cod liver oil, green pasture, or silage, whereas no improvement was noted in others whose ration was supplemented with vitamin D or potatoes.

A more careful study of vitamin requirements was carried out by H. R. Guilbert, C. E. Howell, and G. H. Hart (*J. Nutrition* **19,** *91 (1940)*). Nine colts (Percherons) of both sexes were fed five different vitamin A deficient diets containing 5 to 10 micrograms of the vitamin (measured as carotene) per kilogram of body weight or 0.4 to 1.5 micrograms per kilogram, measured as vitamin A. These were composed of varying quantities of dried molasses, beet pulp, rolled barley, rolled oats, wheat bran, linseed oil meal, cottonseed meal, calcium carbonate, and straw. The animals which received straw ad libitum became night blind in from four hundred and ninety-two to six hundred and twenty-seven days, whereas those receiving no straw were depleted in two hundred and sixty-five to four hundred and thirty-nine days. The difference was ascribed to the presence of carotene in the straw. After the onset of night blindness, continued deprivation of vitamin A resulted, over a period of three or four months, in roughening of the hair, clouding of the corneas, and impaired daylight vision. Excessive lacrimation rather than xerophthalmia was observed. Keratinization appeared, first as streaks, and later involved the entire cornea, but there appeared to be no swelling or elevation of the disc. The horses, although nervous, did not suffer convulsions as observed in cattle. Estrus and libido appeared unaffected. When deficient horses were allowed to die from vitamin A deficiency (C. E. Howell, G. H. Hart, and N. R. Ittner, *Am. J. Vet. Res.* **2,** *60 (1941)*), the night blindness and lacrimation were followed regularly by keratinization of the cornea, reproductive and nursing difficulties, capricious appetite, and progressive weakness. The changes in the hoofs described by Klemola were also present.

Each animal was treated with vitamin A or carotene and depleted repeatedly in an effort to determine the minimum amount of the vitamin necessary to cure and prevent night blindness. It was concluded that 4.2 to 5.3 micrograms of vitamin A (17 to 22 units) or 20 to 30 micrograms of carotene per kilogram of body weight were needed to prevent night blindness. This is in close agreement with the requirements for cattle, sheep, swine, and rats of this vitamin. I. P. Earle (*North Am. Vet.* **23,** *641 (1942)*) pointed out that the requirements for reproduction and lactation are much higher, perhaps up to five times the above figures. A 1200 pound mare, requiring approximately 100,000 units daily, could obtain this amount from approximately 1 pound of alfalfa leaf meal of average quality.

Experiments to determine the vitamin D requirements of the horse do not appear clearcut. Although the statement has been made (C. F. Huffman and C. W. Duncan, *Ann. Rev. Biochem.* **13,** *467 (1944)*) that horses and mules consume enough sun-cured hay to protect them from symptoms of vitamin D deficiency, C. Way (*J. Am. Vet. Med. Assn.* **100,** *335 (1942)*) recorded the impression that thoroughbreds in training may benefit from vitamin D supplementation. This was based upon the frequent observation of skeletal unsoundness in the animal, together with fatigue and general low vitality. This may have been due simply to a calcium-phosphorus imbalance, however, for examination of the blood sugar, calcium, and phosphorus values in 116 such animals revealed that the calcium levels were roughly two thirds of the average figures obtained by four other investigators. Blood sugar levels were likewise low, but the phosphorus values were essentially normal, resulting in a calcium:phosphorus ratio of 2.5:1, compared with a normal ratio of 3:1 or greater. When the normal sugar-calcium-phosphorus level was restored, an improvement in performance and condition was observed.

Way's results (*loc. cit.*) also suggest that vitamin E may be required by the horse.

Twenty barren mares, some of whom had large cystic ovaries, were fed a daily supplement of wheat germ meal during the winter months. At the beginning of the breeding season these animals were reported to have a high percentage of pregnancies. The conclusiveness of this experiment was marred by the fact that an unstated number of animals underwent operations to relieve the ovarian trouble prior to supplementation with wheat germ.

Evidence for the need for ascorbic acid by the horse is somewhat better documented. Since this vitamin had been found to improve breeding performance in cattle, G. K. Davis and C. L. Cole (*J. Animal Sci.* **2,** *53* (*1943*)) administered four 1 g. doses of ascorbic acid to a 3 year old stallion whose semen had become practically devoid of sperm and in which motility was lacking. Following the treatment the sperm count rose to between 2 and 4 billion, and the motility of the sperm rose to approximately 70 per cent activity. When the ascorbic acid was removed the motility decreased to less than 10 per cent within two weeks. Later injection or feeding of ascorbic acid improved semen quality, while cessation of the treatment was followed by decreased quality.

The blood ascorbic acid levels in 12 effective stallions were found to average 126 micrograms per 100 ml., whereas the blood levels in 19 geldings averaged 70. Similar discrepancies were observed among mares, where an unstated number of good breeders averaged 140 micrograms per 100 ml. and the difficult breeders averaged 70. Among the good breeders there was a considerable seasonal variation, which rose from a low of 90 micrograms per 100 ml. in the early spring before green pasture was available, to 170 micrograms during the breeding season (late spring to July 1). A definite correlation thus appears to exist between breeding performance and ascorbic acid levels, although the effect of ascorbic acid feeding to barren mares has been reported with only 2 animals, with one success. Since semen motility decreased rapidly after cessation of ascorbic acid feeding, it would appear that the requirement of this vitamin for normal breeding performance may lie close to 1 g. per day.

Evidence that the horse requires some "B vitamins" was reported by K. Naito, T. Shimanura, and K. Kuwabara (*Third Report, Japanese Government Institute for Vet. Res., p. 51* (*1925*)). These workers fed horses a ration consisting of polished rice and straw supplemented with cod liver oil and minerals. The horses lost weight and developed symptoms characteristic of thiamine deficiency. Later, B. Carlström and A. Hjärre (*Biedermanns Zentr. B. Tiernähr.* **11,** *121* (*1939*)) reported that horses maintained on diets low in "B vitamins" developed anorexia and incoordination of movements. P.B. Pearson, M. K. Sheybani, and H. Schmidt (*J. Animal Sci.* **3,** *166* (*1944*)) expanded these studies in an attempt to discover which of the "B vitamins" was needed. Six groups of 2 Shetland ponies each were placed on a basal diet containing dried beet pulp 63, ground corn 26, purified casein 10, and dicalcium phosphate 1 per cent, respectively. The animals began to lose weight after four weeks (about 20 pounds), and after eight weeks the authors felt that a change in the diet was necessary. Supplementation with 5 per cent yeast retarded the weight loss (about 10 pounds in four weeks), whereas the addition of 10 per cent yeast to the diet resulted in satisfactory growth and good condition of the animals. Since corn is a good source of thiamine and pyridoxine, it was felt that the beneficial effect of yeast was not due to these substances, especially since good growth was obtained when vitamins other than these two were added to the basal diet. Niacin, pantothenic acid, and riboflavin were added and together they promoted excellent growth of the ponies (140 pound gain over a thirty week period).

Studies on the urinary excretion of niacin indicated that either the horse did not need this vitamin or that there was an adequate

concentration in the basal ration. P. B. Pearson and R. W. Luecke (*Arch. Biochem.* **6,** *63* (*1945*)) reduced the niacin intake to 0.01 mg. per kilogram of body weight, or an approximate total intake of 1 mg. per day of the vitamin. Under these conditions the animals excreted approximately 5 mg. per day of the vitamin, and at the same time exhibited no deficiency symptoms. It was concluded that niacin is not a dietary essential for the horse. The horse thus resembles the rat in this respect, although the end products of niacin metabolism are not trigonelline or N′-methylnicotinamide; neither is niacin formed from tryptophan, as has been observed for the rat (*Nutrition Reviews* **5,** *234* (*1947*)).

Urinary excretion studies were used by Pearson, Sheybani, and Schmidt (*Arch. Biochem.* **3,** *467* (*1944*)) to estimate the approximate riboflavin requirements of the horse. Four of the diets used previously by these authors (*loc. cit.*) were supplemented with other vitamins and with riboflavin to contain 0.08, 0.33, 0.36, and 1.18 mg., respectively, per 100 g. of ration. The riboflavin in the second diet was derived principally from yeast, whereas the synthetic vitamin was used in the third diet. The animals who were on the first diet and who received approximately 9 micrograms per kilogram of body weight, did not consume as much feed as the others, and the amount of riboflavin excreted into the urine was so small that it could not be measured. The next two groups which received about 44 and 48 micrograms, respectively, excreted roughly 1.5 mg. per day. Their ratio of intake to outgo was approximately 4:1. The ratio was 2.4:1 on the high riboflavin diet (156 micrograms intake per kilogram), and the authors concluded that the second and third groups had received adequate amounts of riboflavin. Man probably receives 20 to 30 micrograms per kilogram, and when the intake reaches 60 micrograms per kilogram the excretion rises to over 1 mg. per day (W. H. Sebrell, R. E. Butler, J. G. Wooley, and H. Isbell, *Pub. Health Reports* **56,** *510* (*1941*)).

The same approach that was used for studying riboflavin has been used by Pearson and Schmidt (*J. Animal Sci.* **7,** *78* (*1948*)) to estimate pantothenic acid requirements. Essentially the same basal diets were used, containing riboflavin, salt, vitamins A and D oils, and with supplements of pantothenic acid furnishing 3.2, 4.3, 5.4, 6.5, and 9.8 micrograms of this vitamin per gram of feed. All of the diets gave satisfactory growth. The urinary excretion of the vitamin became relatively less as the content in the diet was reduced, so that at the lowest level of pantothenic acid about 13 per cent of the ingested vitamin (38 micrograms per kilogram of body weight) appeared in the urine. At the highest level of intake (150 micrograms per kilogram) 41 per cent were excreted. The authors felt that the pantothenic acid requirements of the growing horse were met satisfactorily by the 38 microgram intake. Such a figure is considerably smaller than the reported requirement of 170 to 235 micrograms per kilogram of body weight of this vitamin for the pig (E. H. Hughes and N. R. Ittner, *Ibid.* **1,** *116* (*1942*)), or for the dog, whose requirement is up to 100 micrograms per kilogram of body weight (A. E. Schaefer, J. M. McKibbin, and C. A. Elvehjem, *J. Biol. Chem.* **143,** *321* (*1942*)). On the other hand, the authors point out that the requirements observed here are similar to those of the rabbit, which like the horse is a herbivore, monogastric, with a large cecum. They report further that rabbits synthesize appreciable amounts of pantothenic acid in the gastrointestinal tract, and it seems possible that the horse may accomplish this also.

Despite the progress which is being made in improving knowledge of equine nutrition, one cannot refrain from expressing the hope at this point that in the future more attention will be paid to direct experimentation and proper documentation of conclusions. The results summarized above with respect

to vitamins D and E, while plausible, need verification. The same is true for thiamine. The conclusions reached with respect to pantothenic acid requirements should be checked by direct experiment with pantothenic acid-free diets, perhaps with the aid of sulfonamides to retard bacterial growth. Information on the remaining water soluble vitamins will be awaited with interest, as well as an extension of knowledge of the interrelations between vitamin D, calcium, and phosphorus in this species.

ACTION OF INSULIN ON ASCORBIC ACID METABOLISM

Despite the large number of investigations on the metabolism of ascorbic acid, little is known of its role in cellular function (*Nutrition Reviews* **4,** *259* (*1946*)). E. P. Ralli and S. Sherry (*Proc. Soc. Exp. Biol. Med.* **43,** *669* (*1940*)) found that administration of insulin to dogs induced a decrease in both the plasma ascorbic acid concentration and in its renal excretion. Further investigation of this phenomenon, with evidence for its mechanism, is now presented by these same workers (*J. Clin. Invest.* **27,** *217* (*1948*)).

The relation between the plasma concentration and ascorbic acid excretion was first studied in some detail in a diabetic dog. Infusions of ascorbic acid and creatinine were given at a constant rate and the clearances of these substances were determined three times in a half hour period. During this time the concentration of ascorbic acid in urine and plasma, as well as the plasma glucose, remained essentially constant. Likewise, there was no significant change in the clearances of ascorbic acid and creatinine, nor in the tubular reabsorption of ascorbic acid. Twenty units of insulin were then administered intravenously and after an interval of half an hour the same determinations were repeated three times in thirty minutes. A prompt fall in both plasma and urinary ascorbic acid concentrations was observed and, in addition, a fall in the ascorbic acid clearance. At the same time there was no change in the renal reabsorption of ascorbic acid. The authors conclude from this that the changes in excretion observed were not due to a primary effect upon the kidneys, but were secondary to the reduction in plasma concentration.

The same changes in plasma and urinary ascorbic acid concentration were induced in further experiments by crystalline, as well as by regular, insulin. Furthermore, the route of administration did not alter the phenomenon, other than to delay and prolong the effect when the insulin was given intramuscularly or, more so, subcutaneously, rather than intravenously.

With the demonstration that the mechanism for the changes was not renal, the authors postulated first that insulin might directly increase the destruction of ascorbic acid or, secondly, prevent its synthesis, or finally that ascorbic acid might be utilized in the oxidation of carbohydrate. In answer to the first, it was shown that insulin added to both whole blood and plasma *in vitro* was without effect upon the ascorbic acid content. The second possibility, that insulin might prevent the synthesis of ascorbic acid, seemed unlikely to the authors because of the rapidity of fall of plasma and urinary values for the vitamin. Moreover, in a normal dog the twenty-four hour excretion of ascorbic acid was not reduced by administration of insulin once daily.

This paradox of normal twenty-four hour excretion despite the acute fall after insulin was explained in further experiments. In these studies it was found that following the ascorbic acid reduction in blood and plasma a rebound existed in which the plasma concentration, and especially the urinary excretion, rose significantly above the control, pre-insulin, levels.

In another series of experiments the twenty-four hour excretion of ascorbic acid in rats was maintained despite daily increasing doses of insulin over a period of eight days. Likewise, in a normal adult male on a constant ascorbic acid intake, from 10 to 45 units of insulin over a fourteen day period failed to reduce the ascorbic acid excretion compared with fore- and afterperiods of nine and fourteen days, respectively. These several experiments indicate that insulin does not act upon ascorbic acid metabolism by increasing its utilization in the body.

The authors had thus disposed of the three postulates to explain the mechanism of action of insulin on ascorbic acid metabolism. There remained the possibility that insulin might induce a shift of ascorbic acid from plasma to other tissues. Indirect evidence in favor of this was found in the observation that, although there was a fall in both plasma and whole blood ascorbic acid, the concentration in the red cells remained the same and there was a rise in the vitamin content of the buffy coat. The rise in ascorbic acid concentration in the white cells, although consistent, did not account for more than a small portion of that lost from the plasma. This led the authors to suggest that the bulk of the plasma ascorbic acid went into body cells, possibly the liver. They concluded from these experiments that the fall in plasma and urinary ascorbic acid following insulin was due to a shift of the vitamin to extravascular areas, possibly to the tissues where the two were concerned together with carbohydrate metabolism.

MINIMUM PROTEIN REQUIREMENTS OF ADULT DOGS

The measurement of minimum protein requirements is not easily accomplished. In determining nitrogen requirements it is recognized that some nitrogen depletion is necessary if minimum requirements are to be obtained. Various investigators have considered it desirable for this purpose to deplete animals to a minimum nitrogen excretion. When an animal is fed a low protein diet, the urinary nitrogen falls rapidly during the first few days and continues to fall for a considerable period but at a decreasing rate. It seems obvious that the amount of protein required to balance these losses will depend upon the time the studies are made or the degree of nitrogen depletion.

In approaching this problem C. F. Wang, A. Lapi, and D. M. Hegsted (*J. Lab. Clin. Med.* **33,** *462 (1948)*) partially depleted dogs of nitrogen as evidenced by urinary nitrogen excretion and plasma protein levels. They were then fed whole egg protein at a level which supplied 2 mg. per basal calory. Nitrogen balance, plasma protein, plasma albumin and hemoglobin concentrations, plasma volume, thiocyanate space, total circulating plasma protein, total circulating hemoglobin, body weight, liver analyses, and microscopic examination at autopsy were used to evaluate the adequacy of the level of protein fed. Their studies showed that, although the amount of nitrogen supplied eventually allowed nitrogen balance, the degree of nitrogen depletion achieved during or prior to the study was sufficiently severe that the results may not be applicable to normal animals.

The study was carried out in 2 male and 4 female adult dogs. Before the experimental period was started, a high protein diet (16 per cent protein) prepared by replacing 44 per cent of sucrose in the basal diet with skim milk powder, was fed for two or three weeks to ensure an optimal nutritional state. The nitrogen-free diet was then given for two or three weeks until the total protein content of the plasma dropped slightly (1 per cent) below the initial level.

The minimum protein level attained in

these studies was thought to be near the theoretical minimum and would correspond to about 20 g. in an average human adult. During the depletion period, from 96 to 230 g. of body protein were lost by the dogs. This was accompanied by a loss of 9.5 to 13.5 g. of circulating plasma protein. The percentage of plasma protein was also decreased slightly but without prior knowledge of the plasma levels it would have been considered to be in the normal range. The point is stressed that these changes occurred before a minimal level of urinary nitrogen excretion was reached. During the next four to six weeks when egg protein was fed, all of the analytic data indicated that the animals were maintained in good condition. The general average showed that nitrogen balance was achieved and the other constituents measured revealed no consistent deterioration. However, microscopic changes were observed in the liver (swelling of the liver cells, marked rarefaction of their cytoplasm, changes in the nuclei) which indicated relatively severe liver depletion.

It is assumed that this degree of depletion was probably achieved during the period on the nitrogen-free diet rather than while the egg protein was being fed. It is of special interest that a barely detectable loss of plasma protein reflected relatively severe liver depletion. It would seem that plasma protein determinations are insufficiently sensitive to evaluate such changes. Wang, Lapi, and Hegsted conclude that the common practice of depleting animals to low nitrogen excretion levels prior to studies on nitrogen requirements is not justified. Levels of nitrogen which apparently maintain such animals in nitrogen balance and also maintain plasma proteins and weight within the low normal range may not be adequate as evidenced by the pathologic changes in the liver and by failure of appetite. They point out that this level of nitrogen may still be adequate to maintain an animal which has not been depleted, especially when methionine is supplied. Evidently further work is required in which nondepleted animals are used to determine whether dietary nitrogen fed at the endogenous level of nitrogen excretion will maintain normal animals.

MALIGNANT MALNUTRITION IN SOUTH AFRICA

Malnutrition in general and pellagra in particular are increasingly serious health hazards in South Africa (*Nutrition Reviews* **5,** *353* (*1947*)). One common and exceedingly complicated nutritional disorder, variously designated malignant malnutrition, infantile pellagra, or Kwashiorkor syndrome, has been discussed in a previous review (*Ibid.* **4,** *303* (*1946*)) and has recently been the subject of reports by J. N. P. Davies (*Lancet* **I,** *317* (*1948*)) and by E. G. Holmes and H. C. Trowell (*Ibid.* **I,** *395* (*1948*)). The conclusions of Davies are of special interest because he relates the syndrome to a disorder of the pancreas induced in some way by nutritional deficiencies.

According to Davies, Kwashiorkor, in more or less severe form, occurs universally in children in Uganda. This is also true of malaria, and it is uncertain to what extent malarial infection intensifies the symptoms. The acute disease is characterized by depigmentation of the skin and hair, alopecia, various skin lesions, macrocytic anemia, edema, decreased plasma albumin and increased plasma globulin, impaired digestion of food, fatty and fibrotic liver, fibrosis of the pancreas, and glomerular hyalinization of the kidneys. In nonfatal cases, recovery is slow, the condition being very resistant to treatment. Adults who have "recovered" are thin and weak, and have soft depigmented hair. Their stools contain undigested food. Anemia and edema are usually

not severe, but these individuals may be thrown back into the acute phase by infection or by shortage of food. Kwashiorkor in some parts of Africa is frequently associated with abnormal iron metabolism and with hemochromatosis, but this is not the case in Uganda.

Examination of hepatic tissue obtained by biopsy and at necropsy from children and adults led Davies to believe that a patchy monolobular fibrosis with accumulation of round cells is the hallmark of Kwashiorkor and that it persists throughout life. Although the fatty liver was one of the earliest lesions noted in biopsy specimens, even in children under 5 years of age, many instances were found in older children and in adults in which fibrosis was present without accumulation of fat. For this reason, Davies believes that the fatty liver was not the initial cause of the observed pathology, but that both the deposition of excess fat and fibrosis in hepatic tissue were secondary to changes in other tissues. Attention was directed to the pancreas when it was observed at routine necropsies that pancreatic fibrosis was extremely common, regardless of age.

According to Davies there is much in the clinical picture of Kwashiorkor to suggest that it is a pancreatic deficiency disease due to malnutrition. The pot-belly, the steatorrhea, the failure to digest meat and starch, and the fatty infiltration of the liver all point to a primary pancreatic dysfunction. In the event of clinical improvement, excess fat disappears from the liver. One curious observation was that swelling of the parotid and other salivary glands, at first thought to be due to mumps, was associated with clinical improvement. Inasmuch as there is also a disappearance of undigested food from the stools, it is possible that there occurred a similar stimulation of the pancreas which contains the same type of tissue as that in salivary glands.

Holmes and Trowell studied the glycogenetic function of the liver in Kwashiorkor patients by means of serial liver biopsies and by blood analyses to determine to what extent essential mechanisms of carbohydrate metabolism were altered. Glycogen determinations demonstrated the presence of appreciable quantities in the liver, the values ranging from 0.8 to 4.8 g. per cent in 15 patients. In 5 control patients, liver glycogen increased within two hours after intravenous administration of glucose, whereas in 8 malnourished individuals the liver glycogen was decreased in all but one. Despite this indication of faulty glycogenesis, the absence of hypoglycemia in most of these individuals, even after fasting for twenty-four hours, indicated that glycogenolysis with maintenance of blood sugar was possible. Sugar tolerance was low, blood sugar values remaining high following either intravenous or oral administration of glucose.

A syndrome which occurs in infants in the West Indies and which is characterized by fatty liver, high mortality, and resistance to ordinary treatment has been described by J. C. Waterlow (*Medical Research Council, London, Special Report Series* **263** (*1948*)). This is believed to be a less complicated form of malignant malnutrition or Kwashiorkor. In the West Indian disease the characteristic hair and skin changes are absent, possibly because ariboflavinosis is not a contributing factor. In addition to the fatty liver, edema, wasting of muscles, reduction of serum proteins, and increased fat in stools were noted. The fatty liver was resistant to choline and methionine but was rapidly returned to normal when sufficient milk was fed. Waterlow ascribed the severity of the disease to prolonged deficiency of protein and overloading with carbohydrate.

The nature of the dietary deficiency in malignant malnutrition is uncertain, doubtless due to variations in associated deficiencies according to food supplies and dietary habits in different localities. The disease does not respond uniformly to purified

nutrients and is refractory in its response to improved diets of natural foodstuffs, even when supplemented with yeast or with liver concentrates. Powdered stomach, for example, has been reported to produce prompt cures (*Nutrition Reviews* **4,** *52* (*1946*)) but was ineffective in the patients studied by Holmes and Trowell. It is probable that the syndrome is the result of multiple deficiencies which may include amino acids, vitamins, and minerals and which may be aggravated by concomitant infection or by toxic dietary constituents. These factors, together with the severity and duration of the malnourished condition undoubtedly determine the degree of reversibility of the biochemical and histologic manifestations of the disease.

Whatever the underlying deficiency may be, it concerns one or more nutrients which are indispensable, directly or indirectly, for the structural integrity and normal functioning of hepatic and pancreatic tissues. Emphasis has been placed on the development of a fatty liver which later becomes fibrotic. Whether the initial failure is in the liver or in the pancreas, as Davies suggests, remains to be determined. The direct involvement of the pancreas is a possibility which merits further study, especially in view of the hepatic degeneration which occurs in dogs following ligation of the pancreatic ducts or extirpation of the pancreas (*Nutrition Reviews* **4,** *187* (*1946*)). Promising studies have already started on the experimental production of malignant malnutrition in animals by dietary means (*Ibid.* **2,** *293* (*1944*); **5,** *353* (*1947*)). The Kwashiorkor syndrome is a challenging problem. Its solution offers the rich reward of alleviation of much human suffering and, in addition, the bonus of significant progress in the understanding of the role of specific nutrients in metabolism.

AVAILABILITY OF PANTOTHENYL ALCOHOL TO HUMAN BEINGS

When the terminal carboxyl group of pantothenic acid is replaced by an hydroxymethyl group, an essentially neutral compound called pantothenyl alcohol is obtained. This compound was apparently first prepared by H. Pfaltz (*Z. f. Vitaminforschung* **13,** *236* (*1943*)), who found that it was approximately as effective as calcium pantothenate in promoting growth and curing the achromotrichia and alopecia of pantothenic acid deficient rats. The explanation for this vitamin activity was apparent when E. Burlet (*Ibid.* **14,** *318* (*1944*)) found that rats and man excreted pantothenic acid in response to administration of pantothenyl alcohol, i.e. the latter compound was converted to pantothenic acid by oxidation in the animal body.

In contrast, pantothenyl alcohol is ineffective in promoting growth of lactic acid bacteria and yeasts which require pantothenic acid; indeed, at high concentrations it interferes with utilization of pantothenic acid by some of these organisms (Burlet, *loc. cit.;* E. E. Snell and W. Shive, *J. Biol. Chem.* **158,** *551* (*1945*)). This inactivity results from the fact that these organisms cannot oxidize the alcohol to the acid, and demonstrates that the acid and not the alcohol is the functional form of the vitamin. The inactivity of pantothenyl alcohol in promoting growth of microorganisms thus permits the determination of pantothenic acid in the presence of pantothenyl alcohol, and this procedure has been followed in the investigations discussed below.

The availability of pantothenyl alcohol to human beings has been further studied by S. H. Rubin, J. M. Cooperman, M. E. Moore, and J. Scheiner (*J. Nutrition* **35,** *499* (*1948*)). After thoroughly establishing the basal excretion levels of pantothenic acid by 7 healthy, adult male subjects on normal rations (2.1 to 5.0 mg. per day, average 3.7

mg.), the urinary excretion of pantothenic acid during twenty-four hours following administration of varying quantities of calcium d-pantothenate was determined. In this way a curve relating extra urinary excretion of pantothenic acid (i. e. excretion over the basal level) to the dose of calcium pantothenate was constructed. In subsequent experiments, the extra pantothenic acid excreted following administration of a test dose of calcium pantothenate or of pantothenyl alcohol could be determined.

Results of two separate experiments showed that the extra pantothenic acid excreted following a test dose of 86 mg. of pantothenyl alcohol was from 107 to 117 per cent of that obtained with an equimolar amount (100 mg.) of calcium pantothenate. When higher doses of the two compounds were fed (215 mg. of pantothenyl alcohol or 250 mg. of pantothenic acid), the extra pantothenic acid excreted following administration of the pantothenyl alcohol was 171 per cent of that excreted following administration of calcium pantothenate.

As usually interpreted, these results would mean that pantothenyl alcohol was actually a more available source of pantothenic acid for man than pantothenic acid itself. It should be pointed out, however, that the test doses administered are much higher than the amounts of the vitamin normally ingested with the ration, and that at the lowest level tested, the "availability" of the two compounds was essentially the same. Comparisons of the relative potencies of the two compounds at concentrations where they are the limiting components of the ration are not available, except for the data of Pfaltz (*loc. cit.*) showing that the two compounds are essentially equally active in rats.

In attempting to explain the "superiority" of pantothenyl alcohol over pantothenic acid in giving rise to excretion of extra pantothenic acid, the authors recalled that R. H. Silber (*Arch. Biochem.* **7**, *329* (*1945*)) had shown that the presence of food in the gastrointestinal tract diminished absorption of calcium pantothenate in dogs. In a repetition of the experiment with human beings, therefore, calcium pantothenate (250 mg.) or pantothenyl alcohol (215 mg.) was given to the subjects in the postabsorptive state, and urinary excretions of the vitamin determined. When excretions of all 7 subjects were averaged, the pantothenic acid output following administration of pantothenyl alcohol was found to be 130 per cent of that following the dose of pantothenic acid. However, in 2 subjects the excretion of extra pantothenate following its postabsorptive administration was unusually low, and when figures for these 2 subjects were omitted from the averages, the "availability" of pantothenic acid and of pantothenyl alcohol was found to be identical for the other 5 subjects.

The authors suggest two hypotheses to explain the greater apparent availability of pantothenyl alcohol when the two compounds are fed at high dosage levels. In the presence of food, pantothenic acid may be lost in the feces more readily than pantothenyl alcohol. It is also possible that pantothenic acid may be destroyed to a greater degree by acid in the stomach than is pantothenyl alcohol. In support of the latter view, they cite unpublished experiments showing that pantothenyl alcohol is considerably more stable than pantothenic acid to acid treatment. A third possibility, not discussed by the authors, is that because of a shorter time of contact with the tissues, a larger percentage of the pantothenic acid which arises by oxidation of pantothenyl alcohol may escape destruction and hence be excreted than is the case when pantothenic acid itself is the compound fed. Because even at these high dosage levels only about 14 per cent of the administered vitamin is excreted unchanged, the remaining 86 per cent having disappeared, this explanation should be considered seriously.

THE EFFECT OF DL-METHIONINE ON THE HEALING OF WOUNDS IN PROTEIN-DEPLETED ANIMALS

Complete starvation was found by E. L. Howes, H. Briggs, R. Shea, and S. C. Harvey (*Arch. Surgery* **27,** *846 (1933)*) to have no influence on the rate of healing in wounds of adult rats. However, in young rats they found that complete or partial starvation did appreciably retard the rate of fibroplasia and thus prolonged the proliferative phase and final healing. A. H. Clark (*Bull. Johns Hopkins Hosp.* **30,** *117 (1919)*) noted that a diet high in protein shortens the lag period in the repair of experimental wounds, while S. C. Harvey and E. L. Howes (*Ann. Surgery* **91,** *641 (1930)*) noted no change in the lag period in protein-fed rats although the velocity of fibroplasia and the proliferative phases were stimulated. W. D. Thompson, I. S. Ravdin, and I. L. Frank (*Arch. Surgery* **36,** *500 (1938)*) found that the hypoproteinemic dog is incapable of normal fibroplasia. Others have confirmed the experimental evidence indicating that protein deficiency is related to faulty healing of wounds and, in protein-depleted human subjects, R. L. Varco (*Surg. Gynec. Obst.* **84,** *611 (1947)*) found that the delayed healing of wounds could be corrected by administration of high protein diets and hydrolysates.

Because of the evidence that the sulfhydryl (S H) group favors growth and because it has been shown that in rats and dogs DL-methionine spares nitrogen, S. A. Localio, M. E. Morgan, and J. W. Hinton (*Surg. Gynec. Obst.* **86,** *582 (1948)*) undertook to study the effect of this essential amino acid on the healing of wounds.

Young white adult male rats of pure Wistar strain were used. There were 65 normal controls and 63 animals which were depleted and rendered hypoproteinemic by means of diet. A third group of 67 animals was depleted in the same way but after five weeks of depletion and during the course of wound healing these animals received DL-methionine. Aseptic wounds exactly 3 cm. in length were made to the right of the midline in the upper abdomen of the animals. The wounds were closed with interrupted sutures of stainless steel wire and successive animals were killed daily after one to ten days, the tensile strength of the wounds being determined by distending the peritoneal cavity with air and measuring the bursting pressure of the wound in millimeters of mercury according to a technic previously described by these workers (S. A. Localio, W. Casale, and J. W. Hinton, *Surg. Gynec. Obst.* **77,** *243 (1943)*). The first group of animals was maintained on a basal diet of Purina chow, while the second and third groups were placed on a diet consisting of carrot powder, Crisco, agar, and salt. Vitamins were not added to the diet. The diet contained 0.23 per cent of nitrogen. Animals in the deficient group were maintained on this diet for five weeks and were then operated on. Those animals which received methionine were given 150 mg. dissolved in sterile, distilled water and administered subcutaneously daily for a period of ten days. The last injection of methionine was given twenty-four hours before the animal was killed, but the time when the animals were given this amino acid varied from eight days before the experimental wound was produced to the day of production of the experimental wound.

Protein depletion in rats was associated with a prolonged lag period, a slow proliferative period, and delayed final healing. The parenteral administration of DL-methionine to protein-depleted rats shifted the curve of wound healing toward normal. The lag period was decreased and final healing was accelerated despite continued protein depletion. In attempting to explain these results, Localio and his associates point out that in depleted animals wound healing ultimately does occur. Consequently re-

generation must take place at the expense of other cells of the organism. It would seem plausible, therefore, to assume that the DL-methionine supplies to the wound some factor that is not readily available, or not available in sufficient quantities in the materials which the organism is able to supply readily to the healing wound. As a result, a significant improvement in healing can take place even in the face of general protein deficiency. If the sulfhydryl radical is the chemical group that the depleted organism cannot supply the healing wound in sufficient quantities, as they suggest, and if without it the lag period of wound healing is delayed, then a local wound function for the sulfhydryl radical may be postulated. The sulfhydryl enzymes may exert their most important function during the early stages of healing. Without this enzyme activity the lag period of healing is prolonged; with it, it is shortened.

Experiments are under way to test the effect of other amino acids and of sulfhydryl groups derived from other compounds on wound healing.

EFFECT OF ADMINISTERED IRON DURING PREGNANCY

Much has been written about the so-called physiologic anemia of pregnancy. The favorable effects of many therapeutic substances have been both claimed and denied. Two studies made in Britain during World War II make possible an evaluation of the significance of the hemoglobin levels of pregnant women in England and aid in understanding the nature of the "physiologic anemia of pregnancy."

The report of the Committee on Haemoglobin Surveys of the Medical Research Council (*Medical Research Council, London, Special Report Series* **252** *(1945)*; *Nutrition Reviews* **4,** *154 (1946)*) included observations on 690 pregnant women in attendance at antenatal clinics. These data indicated that the mean hemoglobin level decreased significantly and progressively from the first to the third trimester of pregnancy—the successive mean values for the three periods were 89, 86, and 82 per cent (100 per cent = 13.8 g. of hemoglobin). There was a further suggestion that a rise in hemoglobin concentration might occur at the end of pregnancy, but the data were insufficient to prove this point. This survey did not permit a conclusion as to the normal hemoglobin level during pregnancy or as to whether the values found could have been increased by providing the pregnant woman with a more generous quantity of various hematopoietic substances. Other data collected by the Committee, however, appear related to these questions. Observations on nonpregnant women were analyzed by parity, and parous women were found to have lower mean hemoglobin levels than did nulliparae. Furthermore, the hemoglobin values of married women were found to decrease progressively according to the number of previous pregnancies. These observations indicated an increased need for hematopoietic factors proportional to the parity of the woman. Such a need might, of course, be due directly to parturition or indirectly to a lower nutritional state in the mother as the size of the family increased.

Employing the same methods as used by the Committee on Haemoglobin Surveys, L. Wills and co-workers (*Brit. J. Nutrition* **1,** *126 (1947)*) report observations on 500 unselected women attending the antenatal clinic of the Royal Free Hospital in London. Alternate patients were given, from the first visit, either capsules of ferrous carbonate (equivalent to 0.58 g. of iron daily) or a similar capsule which contained no iron. Excluded from the study were 9 women who had hemoglobin values below 70 per cent associated with the blood picture

of a hypochromic (iron deficiency) anemia at their first visit.

The two groups of patients had comparable initial hemoglobin levels—89.77 ± 0.34 per cent. The mean levels for the iron supplemented and placebo groups respectively were 85.2 ± 0.52 and 83.5 ± 0.51 per cent in the second and 86.4 ± 0.51 and 80.7 ± 0.54 per cent in the third trimester. Similar differences between the mean values of the two groups continued through the six to twelve week postpartum check—the iron supplemented women invariably exhibited higher mean hemoglobin levels.

These well presented data demonstrate that the routine administration of iron during pregnancy was followed by significantly higher hemoglobin levels than found in a comparable control series. Despite the iron, however, there occurred a significant decrease in hemoglobin concentration in the treated group until the thirty-second week of gestation. It is not possible to state whether this decrease in the presence of added iron should be termed a "physiologic anemia" (due to hydremia) of pregnancy or whether it in turn reflects a need for other hematopoietic agents in amounts greater than supplied by the diets of these women.

Blood counts and hematocrit determinations (estimation of packed-cell volume) were made on 44 of the women. Except in 2 cases, the red cell counts decreased during pregnancy, but this drop was stated to be "considerably smaller" in the treated than in the untreated group. The mean corpuscular volume remained constant or increased slightly as pregnancy progressed—no value below 80 microns^3 was observed. This slight tendency toward an increased corpuscular volume in pregnancy has been noted by other workers (R. K. Anderson, W. D. Robinson, J. Calvo, and G. C. Payne, *J. Am. Dietet. Assn.* **22,** *588 (1947)*). Its significance, if any, has not been defined. It would have been valuable to have data on mean corpuscular volume and mean corpuscular hemoglobin concentration on all of these subjects to test whether it would be possible to categorize the group of patients who exhibited hemoglobin effects after iron therapy.

The incidence of toxemia, puerperal sepsis, and maternal or infant mortality was low in both groups and no difference in these was found between the unsupplemented and the supplemented series. Furthermore, no difference was found in the mean birth weight of the two series. The mean estimated blood loss was 29.0 and 38.5 ounces for the treated and untreated groups, respectively. Although this difference is significant statistically, it is to be noted that estimates of blood loss at labor are difficult and must be recognized for the qualitative evaluations which they represent.

Wills and co-workers conclude that routine administration of iron to pregnant women seems justified. Such a conclusion is in keeping with the "protective nutrition therapy" as is frequently recommended during pregnancy (J. B. Youmans, "Nutritional Deficiencies," *J. B. Lippincott Co., Philadelphia (1941)*). However, the demonstrated benefit on this group of English women during wartime cannot necessarily be generalized to all groups of pregnant women. More studies of this point are required to define the groups of women who are benefited by added iron and, in addition, to ascertain whether the iron is required to meet a deficiency which develops during pregnancy or which existed prior to the pregnancy. The results of the studies on nonpregnant parous women lead to the suggestion that the latter may be the case. If so, the iron loss may occur through the postpartum bleeding and, therefore, the logical time for iron supplementation may be postpartum rather than during pregnancy. In this connection, it is to be recalled that some estimates of iron requirements during pregnancy have not placed them higher than the quantity of iron which would be lost during the menstrual periods missed during the pregnancy.

BACTERIAL UTILIZATION OF PEPTIDES

Peptides recently became of considerable interest in the field of nutrition when a peptide which was found to contain glutamic acid and which was named strepogenin by the investigators, H. Sprince and D. W. Woolley (*Nutrition Reviews* **4,** *273 (1946)*) was demonstrated to be a growth factor for a number of microorganisms as well as for mice. Then it was demonstrated that a material which caused wilting of tomato leaves called lycomarasmin was a peptide containing aspartic acid and which, by reason of its structural similarity to strepogenin, inhibited the activity of strepogenin (*Ibid.* **5,** *218 (1947)*). In rather direct contrast to the strepogenin studies, the amino acids comprising the peptides which occur in natural materials such as plasma or urine were only partially available for the growth and acid production of lactic acid bacteria. Lower values for the component amino acids were obtained when determined by bacteriologic assay than when amino acids were determined after hydrolysis by chemical procedures (*Ibid.* **5,** *213 (1947)*).

To determine the extent to which leucine in certain peptides could be utilized by lactic acid bacteria, W. A. Krehl and J. S. Fruton (*J. Biol. Chem.* **173,** *479 (1948)*) conducted a series of investigations using the following microorganisms from the American Type Culture Collection: Lactobacillus arabinosus 8014, Lactobacillus casei 7469, Streptococcus faecalis 9790, and Leuconostoc mesenteroides P-60 8042. The basal medium used was comparable to that used in most microbiologic assays for amino acids and contained all the amino acids known to be needed for the growth of the lactic acid bacteria in as pure form as could be obtained. In addition, adequate amounts of salts, glucose, and the necessary vitamins and growth factors were present in each tube. All assay tubes were incubated at 37°C. and turbidity measurements made with the Evelyn photoelectric colorimeter (using filter No. 620) at appropriate intervals. At the end of the seventy-two hour incubation period, acid production was determined by titration with tenth-normal sodium hydroxide. To determine the ability of L. arabinosus and S. faecalis to utilize the L-leucine in a series of peptides, a series of standards was run to determine the amount of tenth-normal acid formed per tube, after seventy-two hours incubation in the presence of varying amounts of L-leucine, both in the presence and in the absence of D-leucine. In addition, two samples of DL-leucine were tested for their ability to promote growth of these two organisms. One of several levels of L-leucine between 0 and 100 micrograms was added to each of a series of tubes to produce a standard curve of the response of the microorganisms to varying amounts of L-leucine. In addition, turbidity measurements were made on the standardization tubes at the end of sixteen and forty hours. With the DL-leucine, sufficient was added that the above amounts of L-leucine would be present in each tube. In making the tests on the peptides and derivatives containing L-leucine, sufficient of the compound to supply some amount of L-leucine between 20 and 100 micrograms per tube was added, so that the degree of turbidity at intermediate times in the incubation period and the value of tenth-normal acid produced by each tube would fall somewhere along the standard curve. Altogether seventeen peptides and derivatives of L-leucine and D-leucine were tested for both organisms. All of the peptides of L-leucine which were tested served to some degree as growth factors for the two organisms employed in these studies. None of the peptides containing D-leucine had any appreciable growth-promoting activity. At seventy-two hours incubation, utilization of most of the peptides was considerably less than that to be predicted if all of the leucine present in the form of peptides had been available for growth. In addition, there was an appreciable variation in the response of

the two organisms to individual peptides. With the L. arabinosus, L-leucine-L-tyrosine was found to be completely available. Likewise, the L-leucine in this compound was almost completely available for growth of S. faecalis. However, in the case of glycyl-L-leucine, the L-leucine was completely available for L. arabinosus, but apparently only about 72 per cent available for S. faecalis. All derivatives of D-leucine were uniformly inactive as was the D-leucine which was added in the preparation of standard curves, but the presence of D-leucine did not interfere with growth. Leucine derivatives such as N-methyl-DL-leucylglycine, acetyldehydroleucine, acetyldehydroleucylglycine, and acetyldehydroleucinamide were completely inactive for both organisms, and only slight activity was noted for acetyl-L-leucine in the case of L. arabinosus. An important observation was made in terms of the utilization of the L-leucine in DL-leucine. One sample was found to have had less than the theoretical 50 per cent activity, while the other preparation permitted the same amount of growth as an equivalent amount of L-leucine. This difference was undoubtedly due to the presence of impurities in the first sample, indicating the need for careful standardization of all amino acids to be used in studies of this type.

Krehl and Fruton (*loc. cit.*) tested the activity of L-serylglycyl-L-glutamic acid for strepogenin activity, since D. W. Woolley (*Nutrition Reviews* **5**, *218 (1947)*) had reported that a mixture of the diastereo-isomeric D- and L-serylglycyl-L-glutamic acids had strepogenin activity. In confirmation of the previous investigation, Krehl and Fruton observed that L-serylglycyl-L-glutamic acid had definite strepogenin activity for L. casei. In addition, the very similar peptide L-seryl-L-alanyl-L-glutamic acid was tested and found to have no strepogenin activity whatever for L. acidophilus under the conditions of their assay. Likewise, alpha-L-glutamyl-L-glutamic acid, alpha-L-glutamylglycylglycine, diglycyl-alpha-L-glutamylglycine, and glycyl-alpha-L-glutamyl-L-tyrosine were entirely inactive. Using liver fraction L as a source of strepogenin, the investigators tested whether other lactic acid bacteria needed strepogenin for growth, and of their four test organisms only L. casei and L. mesenteroides P-60 were found to require strepogenin as supplied in liver fraction L.

These experiments on the utilization of various peptides and derivatives of L-leucine demonstrate that the growth-promoting activity of such peptides depends a great deal upon the position of the leucine residue with respect to the other amino acid residues, the nature of the other amino acids, the test organism used, and the conditions and length of time of the incubation. In view of these wide variations in the growth-promoting activity of amino acid-containing materials, the need for careful and complete hydrolysis of peptides in all natural material becomes evident. At the same time, the reverse phenomena of peptides stimulating the growth of bacteria becomes a complicating factor. At the present time only the one peptide has been described as having strepogenin activity and the series of reasonably closely related ones which have been tested have had no stimulatory activity. However, it is not known at the present time whether the activity of liver fraction L as a growth-promoting factor for certain bacteria, is due to the peptide L-serylglycyl-L-glutamic acid described by Woolley and tested by Krehl and Fruton or whether there may be additional ones contributory to the stimulatory effect.

DIET AND TUBERCULOSIS SUSCEPTIBILITY IN MICE

The importance of providing an adequate diet for patients with pulmonary tuberculosis is firmly established. Moreover, the epidemiologic association of malnutrition and

phthisis has suggested that improper or insufficient food may be an important etiologic factor in the disease in man. The experiments reported by R. J. Dubos and C. Pierce (*Am. Rev. Tuberc.* **57,** *287* (*1948*)) are in agreement with the clinical conclusions and may form a beginning for determining which, if any, of the specific nutrients is of importance.

The susceptibility to tuberculosis of mice maintained on three different diets was studied. Three strains of mice were used, line "dba," Swiss albino, and Rockefeller Institute albino. They were maintained during a foreperiod of at least a week on a diet of white bread soaked in milk. Inoculation intravenously or intracerebrally was then performed with a mammalian tuberculosis strain and at the same time the diet was changed to one of three experimental ones. The first of these contained 100 g. of corn meal and 5 g. of butter, the second was the same as the first except that 100 ml. of 10 per cent gelatin were added. The third, "Sherman 100," was made of 66 per cent ground whole wheat and 33 per cent whole milk. Salt mixtures were added to all diets. A group of animals was continued on the bread and milk regimen during each experiment, and for each diet a set of uninfected controls was kept.

None of the uninfected animals of the dba strain on the corn meal-gelatin diet died and, in fact, they grew nearly as well as did those on the bread and milk regimen. In contrast, of the infected group on this diet, 7 of 8 animals were dead within thirty days with an average survival time of twenty-nine days, while the infected animals on the bread and milk diet survived for an average of fifty-eight days. The Swiss albino mice on the corn meal-gelatin ration likewise fared much worse than the animals fed bread and milk. Further experiments showed that deletion of gelatin did not significantly change the survival time, nor was the route of infection important (i.e., intravenous or intracerebral).

Similar experiments demonstrated that although the bread and milk diet gave far more protection than the corn meal ration, the Sherman 100 diet offered the best chance of mouse survival.

Realizing that the corn meal-gelatin diet is a deficient one, supplements of tryptophan, tyrosine, lysine, niacin, and yeast extract were given singly or in various combinations. The authors state that no increase in survival time was achieved with these supplements; in fact, the opposite appeared to be the case in some instances. It is well known that diets supplemented with amino acids are often poorly eaten. One wonders whether this could account for the failure of supplementation to prolong survival. However, as the authors point out, the possibility must be considered that the diet contains a substance enhancing the infection, quite apart from dietary deficiency. The pellagragenic factor described by D. W. Woolley (*Nutrition Reviews* **5,** *163* (*1947*)) might act by increasing susceptibility in this way. Whatever the explanation for the results observed, experiments such as these open the way for further study of the relation of nutrition and resistance or susceptibility to infection.

HUNGER EDEMA IN CHILDREN

Edema due to malnutrition was frequent among the starving populations of Europe during World War II (*Nutrition Reviews* **3,** *95*, *225* (*1945*)). E. P. Petrides (*J. Pediatrics* **32,** *333* (*1948*)) has reported a study of 106 children in Athens who had hunger edema during 1941 to 1943. Edema appeared first in older age groups, especially manual laborers, then in children, the incidence being high between the ages of 1 and 3 years, and greater in males than in females. No edema was seen in infants under 6

months of age, due presumably to death when a basal amount of human milk was unavailable. Clinical findings in the malnourished children included pallor, marked emaciation, muscle wasting, delayed development, prostration, and edema. Swelling appeared first over the dorsal surfaces of the feet and ankles, then the hands and eyelids, and finally progressed to massive anasarca. Ascites, hydrothorax, and hydropericardium were common. Body weight was diminished despite edema. Hypothermia, bradypnea, basal pulmonary rales, bradycardia, distant heart sounds, and hypotension were usual findings. Furunculosis, fine desquamation of the skin over the chest, gastrointestinal disturbances, and polyuria were often noted. Xerophthalmia was noted in 3 children, keratomalacia in one, hemorrhagic macules and bruises in 7, and pellagrous dermatitis in 4. Mantoux tests in dilution of 1:10,000 were positive in 24 children. Prognosis depended on the degree of malnutrition, the possibility for correction, and existing complications. The mortality was 24 per cent.

Laboratory studies showed anemia, leukopenia with lymphocytosis, low blood urea, and severe hypoproteinemia. The relation of total serum protein concentration to edema was investigated in 40 children. Serum proteins were measured by the refractometric method of T. B. Robertson (*J. Biol. Chem.* **11,** *179 (1912)*) which is nonspecific and may be influenced by the concentration of urea, lipoid, sugar, and other substances in the blood. Findings should accordingly be interpreted with caution. The normal level of serum proteins was considered to be at least 5.9 g. per 100 ml. of blood. In 28 of 30 children, serum proteins were low, ranging from 2.8 to 5.8 g. per cent. Average values were 4.42 g. in 12 patients during the first day in the hospital, 4.37 g. in 13 children during the second day, and 4.94 g. in 5 children during the third day. The higher values were found in children less than 3 years of age, which the author attributes to a larger intake of animal protein in the form of milk.

Thirty-three children were given increasing amounts of food each day and serum proteins determined repeatedly. Values rose gradually reaching normal in an average of eleven to twelve days. There was a parallel decrease in body weight with disappearance of the edema in thirteen to fourteen days.

The effect of animal protein on hunger edema was investigated in 4 children. In 3, no animal protein was given for thirteen, sixteen, and nine days respectively, the diet being what the author designates "complete" in terms of other foods and calories. Vitamin supplements were administered. Serum proteins were relatively unaffected in the first 2 children and decreased from 4.9 to 3.9 g. per 100 ml. in the third. Body weight and edema decreased somewhat in the first child, remained stationary in the second, and increased in the third. After the above periods of observation, milk, eggs, and cream were added to the diet at the expense of carbohydrate and fat, while the caloric intake remained constant. A rapid rise in serum proteins and loss of edema followed.

The fourth patient was given a high caloric diet which was high in vegetable protein but free of animal protein. After seven days an increase in serum proteins was observed, a concentration of 6.98 g. being attained in fourteen days at which time edema had disappeared. The diet was continued, and between the fifteenth and twenty-third days there was a slight decrease in serum proteins and a concomitant increase in body weight. Reduction of caloric intake and the addition of animal protein to the diet were followed by a rise in serum proteins to 8.12 g.

Unfortunately the diets used in these studies were not described in detail either in terms of foodstuffs or of individual nutrients. This information would be of great assistance in the evaluation of findings.

In 3 children the effect of special animal proteins on re-establishment of normal levels

of serum protein was investigated. One patient received milk and sugar in amounts sufficient to satisfy caloric needs and to furnish twice the protein requirement. Serum proteins increased steadily and edema disappeared within eleven days. A second patient, aged 2 years, was given ten egg whites and enough sugar daily to supply adequate calories. Serum proteins remained essentially constant for twelve days while the weight decreased slightly. A normal diet on the thirteenth day was followed almost at once by disappearance of edema and a rapid rise in serum proteins. The third patient, aged 2½ years, received five egg yolks and 75 g. of sugar daily which was considered adequate in calories, protein, carbohydrate, and fat. There was a continuous increase in serum protein, a decrease in body weight, and a loss of edema within twelve days.

Nutritional edema was produced experimentally in 3 normal children with a diet which contained small amounts of vegetable protein, no animal protein, more than adequate fat, and sufficient calories. Vitamin B-complex was given parenterally. Total serum proteins were 7.4, 7.4, and 8.5 g. at the beginning of the study. Subnormal concentrations (5.0, 5.2, and 5.3 g.) were found on the thirty-eighth, twenty-fourth, and twentieth days, respectively. During this period body weight decreased slightly, followed by an increase prior to the appearance of edema on the thirty-ninth, thirty-seventh, and twentieth days. At the end of forty to forty-one days, animal protein was added to the diet, and six to seven days later serum proteins were normal and edema had disappeared. It is regrettable that again no details were given concerning the diets used in this study.

The author's findings are interesting, but do not justify his conclusion that nutritional edema is due to hypoproteinemia with resultant low osmotic pressure. He showed a close, but not necessarily causal relation between hypoproteinemia, as determined by the refractometric method, and edema. He also demonstrated that a high caloric intake alone was not efficacious in treating famine edema and that sufficient protein, particularly of animal source, must be included in the diet.

Findings at variance with those of Petrides have been reported by A. Keys, O. Mickelsen, and A. Henschel (*Science* **103,** *669 (1946)*; *Nutrition Reviews* **5,** *31 (1947)*) who concluded that recent investigations, including their own studies of men maintained on a European-type of famine diet, fail to substantiate the simple hypoproteinemia theory of famine edema. Keys *et al.* suggested that famine edema is not always or even frequently explicable by the Starling equation and hypothesized "a dynamic *non* equilibrium state of the capillary wall." A complete understanding of the pathogenesis of hunger edema awaits further investigation.

COMPARATIVE BIOLOGIC ACTIVITY OF BETA-CAROTENE AND VITAMIN A

Following demonstration of the structural relation between beta-carotene and vitamin A, Karrer postulated that in the conversion of beta-carotene to vitamin A in the animal body, symmetrical cleavage occurred to produce two molecules of the vitamin from one of beta-carotene. According to this view, vitamin A and beta-carotene would be equally active on the weight basis. This idea was generally accepted until vitamin A became available in crystalline form. Assays of the crystalline vitamin then showed that it contained from 3 to 4 I.U. of activity per microgram, whereas beta-carotene, by definition, contained only 1.67 I.U. per microgram. Thus, vitamin A alcohol appeared to be slightly over twice as potent as beta-carotene. The theory therefore became current that beta-carotene could not be converted into vitamin A by symmetrical fission,

but must be attacked in some random fashion which gave rise to only one molecule of the vitamin per molecule of beta-carotene decomposed, so that on a weight basis, beta-carotene should be only about one-half as active as vitamin A.

Subsequently, the beneficial effects of vitamin E on utilization of vitamin A were noted, and data were obtained which indicated that the optimum level of tocopherols necessary to ensure maximum usage of beta-carotene was considerably higher than that required for optimum utilization of vitamin A alcohol or its acetate (K. C. D. Hickman, *Ann. Rev. Biochem.* **12,** *353 (1943)*).

Because of these conflicting results, C. J. Koehn (*Arch. Biochem.* **17,** *337 (1948)*) has redetermined the relative biologic potencies of pure vitamin A alcohol, its acetate, and beta-carotene under identical dietary conditions and in the presence of adequate alpha-tocopherol. These compounds were administered to vitamin A-depleted rats daily, in a medium which also supplied 1.0 mg. of alpha-tocopherol per day per rat. Special precautions were taken to prevent any destruction of the active compounds before their actual ingestion by the rat. The results showed unequivocally that beta-carotene and vitamin A alcohol had equal activities on the weight basis.

These results thus once more support the original theory of Karrer that conversion of beta-carotene into vitamin A *in vivo* is by fission of the molecule to yield two molecules of vitamin A. Thus the recently achieved chemical fission of beta-carotene to two molecules of vitamin A aldehyde, followed by reduction of the latter to the alcohol (*Nutrition Reviews* **5,** *64 (1947)*) may provide a model for the manner in which this reaction is accomplished *in vivo*.

The bearing of these results on those of other workers is discussed. From the available evidence, and partially because some assay diets have contained inadequate amounts of tocopherols, it appears that the vitamin A activity of beta-carotene has been generally underestimated in the past, and also that the U.S.P. reference oil (standard for vitamin A determinations) has contained less than the stated amount of vitamin A. These factors combine to make many data in the literature on the vitamin A content of foodstuffs of questionable accuracy.

EFFECT OF ALLOXAN ON NORMAL AND NEOPLASTIC PANCREATIC ISLET CELLS IN MAN

J. W. Conn and D. L. Hinerman (*Am. J. Path.* **24,** *429 (1948)*) have described a patient with an islet cell tumor of the pancreas, many of the islet cells of which upon surgical removal of the tumor were found to have been destroyed by a previous period of alloxan administration. Although this one case does not constitute a thorough investigation, it is one of importance to nutrition investigators since it demonstrates the similarity of action of alloxan upon the pancreatic islet cells of man to its effect on these cells in experimental animals.

On admission to the hospital, this patient was found to have a severe hyperinsulinism, a very high tolerance to carbohydrate, and a low fasting blood sugar. Administration of alloxan on nine consecutive days for a total of 800 mg. per kilogram of body weight resulted in a slight increase in fasting blood sugar and a reduction in tolerance to carbohydrate which continued a few days after alloxan administration was stopped. The persistence of hypoglycemic attacks made necessary surgical removal of an islet cell tumor. Within twenty hours after removal, a frank diabetes developed with heavy glycosuria, ketonuria, and a fasting blood sugar level of 208 mg. per cent. Insulin therapy for about four weeks resulted in control of

the diabetic syndrome; insulin was then diminished gradually until repeated glucose tolerance tests after cessation of insulin therapy indicated a return to almost normal.

Examination of the neoplastic islet cells and of normal cells from adjacent pancreatic tissue indicated a definite sensitivity of the islet cells to alloxan. The changes in the islet cells varied from mild degenerative changes to a disappearance of recognizable cells. In some 7 per cent of the islets, a marked hyperplasia with brilliant staining of the newly formed cells was observed. The sensitivity of normal human islet tissue was found to approximate that of other species. However, in this patient, neoplastic islet tissue was much more resistant to the injurious effect of alloxan than normal islet tissue which would suggest the presence of different metabolic processes in neoplastic islet cells. The alpha and delta cells of human islet tissue are susceptible to damage but to a much lesser degree than are beta cells. Because of the resistance of neoplastic islet cells, the level at which alloxan would have to be administered to produce a sufficiently destructive effect on the neoplastic islet cells to be valuable as a therapeutic measure seemed to be likely to be lethal to the patient. However, this difference in resistance of normal and neoplastic cells may prove to be a valuable approach to the investigation of the difference in metabolic processes of the normal and pathologic cells.

THE STRUCTURE AND ACTIVITY OF PTEROYLGLUTAMATES

At the time of the announcement of the structure of liver folic acid (pteroylglutamic acid) evidence was presented by E. L. R. Stokstad and co-workers (*Ann. N. Y. Acad. Sci.* **48,** *269 (1946)*) that the fermentation Lactobacillus casei factor contained three molecules of glutamic acid combined in peptide linkage to pteroic acid. J. J. Pfiffner, D. G. Calkins, E. S. Bloom, and B. L. O'Dell (*J. Am. Chem. Soc.* **68,** *1392 (1946)*) reported that vitamin Bc conjugate contained 7 glutamic acid units, presumably similarly combined. No syntheses of either compound were given, so that the exact structure, including the type of linkage of the glutamic acid residues, remained a matter of conjecture.

Two papers from the Lederle laboratories (J. H. Mowat *et al.*, *J. Am. Chem. Soc.* **70,** *1096 (1948)*; J. H. Boothe *et al.*, *Ibid.* **70,** *1099 (1948)*) reveal that the glutamic acid molecules are joined in gamma-peptide linkage (i.e., through the gamma-carboxyl group) in both the fermentation factor and in pteroyldiglutamic acid, a biologically active synthetic product which is unknown in nature. To establish these structural relations, pteroyldiglutamic acid was first synthesized containing the more easily prepared alpha-linkage. This compound possessed less than 1 per cent of the activity of pteroylglutamic acid for L. casei and Streptococcus faecalis. Pteroyl-gamma-glutamylglutamic acid, on the other hand, was 60 to 70 per cent as active. Similarly, pteroyl-gamma - glutamyl - gamma - glutamylglutamic acid possessed the full activity of the natural fermentation factor.

Similar information has not been presented for Bc conjugate. However, in view of these experiments it seems reasonable to suppose that the gamma-linkages also prevail in this compound, presumably in a linear peptide (branching of the tripeptide chain in the fermentation factor destroys the biologic activity). If this is true, it would suggest that the gamma-linkage may be the common one in other glutamic acid peptides as is known to be the case with glutathione.

Closely related to the problem of the structure of the various folic acid conjugates is that of their degradation by conjugases. Crude Bc conjugase (O. D. Bird and coworkers, *J. Biol. Chem.* **157,** *413 (1945)*) was

obtained from hog kidney or chicken pancreas. It was found to convert the heptaglutamate, which showed no microbiologic activity *per se*, into an active form of the vitamin. A. Kazenko and M. Laskowski (*Ibid.* **173,** *217 (1948)*), in a more extensive study of this conversion, tested the activity of a relatively purified conjugase from chicken pancreas upon a number of related peptides. Glutathione and those peptides possessing alpha-glutamic linkages were unaffected by the conjugase, whereas para-aminobenzoyl - gamma - glutamyl - gamma - glutamylglutamic acid and the natural and synthetic fermentation factors were attacked, yielding free glutamic acid. However, the data indicated that only the terminal glutamic acid was removed; the degradation process appeared to stop at this point, leaving (in the case of the fermentation factor) pteroyldiglutamic acid. It seems likely that in the determination of the folic acid content of natural materials after digestion with chicken pancreas, the diglutamate may represent a part of the active material measured. Whether the pure chicken pancreas conjugase is able to degrade Bc conjugate to a compound with full folic acid activity for various species is not yet known. Further study of this point with conjugases from different sources might help to throw light upon the peculiar situation which exists in the protozoon Tetrahymena gelii, in which the heptaglutamate is approximately twice as active as the monoglutamate (*Nutrition Reviews* **6,** *38 (1948)*), or upon the problem of the inconstant effectiveness of the heptaglutamate in human pernicious anemia in relapse (F. H. Bethell *et al.*, *J. Lab. Clin. Med.* **32,** *3 (1947)*; E. A. Sharp and E. C. Vonder Heide, *Am. J. Clin. Path.* **17,** *761 (1947)*). In any event, the biologic role of the pteroylpolyglutamates seems closely related to the gamma-peptide structure.

On the other hand, there is considerable evidence that at least one glutamic acid residue must be in the molecule for these pterin derivatives to possess biologic activities for most species. For example, both pteroic acid and rhizopterin (formylpteroic acid, see *Nutrition Reviews* **6,** *211 (1948)*) are inactive for L. casei and for man.

Other studies on the structural specificities of these compounds have been reported. Alteration of the pteridin configuration by introduction of a 7-methyl group (*Nutrition Reviews* **6,** *23 (1948)*) or substitution of an amino group for the 4-hydroxy (D. R. Seeger, J. M. Smith, and M. E. Hultquist, *J. Am. Chem. Soc.* **69,** *2567 (1947)*) of pteroylglutamic acid results in compounds antagonistic to folic acid (A. L. Franklin, E. L. R. Stokstad, and T. H. Jukes, *Proc. Soc. Exp. Biol. Med.* **67,** *398 (1948)*) and without hematopoietic activity for man (T. D. Spies *et al.*, *Blood* **3,** *121 (1948)*).

The replacement of the pteridin nucleus of PGA by a quinazoline ring yielded a compound which possessed up to 10 per cent of the activity of folic acid for S. faecalis (G. J. Martin, J. Moss, and S. Avakian, *J. Biol. Chem.* **167,** *737 (1947)*). A similar replacement of the pterin by benzimidazole resulted in an analogue with roughly 0.001 per cent of the activity of PGA (P. C. Edwards, D. Starling, A. M. Mattocks, and H. E. Skipper, *Science* **107,** *119 (1948)*).

Such studies as these will eventually help clarify the metabolic role of the pteroylglutamates and the enzymes which act upon them and, possibly, result in the development of new chemotherapeutic agents.

DIETARY IMPAIRMENT OF ESTROGEN RESPONSE IN THE IMMATURE MONKEY

The relation between dietary deficiencies and estrogen activity has been discussed previously in this journal (*Nutrition Reviews* **3,** *308 (1945)*). In particular, the results of

R. Hertz and W. H. Sebrell (*Science* **100,** *293* (*1944*); Hertz, *Endocrinology* **37,** *1* (*1945*)) which showed that folic acid (pteroylglutamic acid) was necessary to permit the growth response of the chick oviduct to stilbestrol injections were emphasized.

In attempting to determine the generality of this relation, Hertz (*Proc. Soc. Exp. Biol. Med.* **67,** *113* (*1948*)) placed 10 immature female monkeys on a synthetic diet deficient in folic acid. This diet was comparable to the vitamin M deficient diet of P. L. Day, W. C. Langston, and W. J. Darby (*Ibid.* **38,** *860* (*1938*)), on which monkeys show a good therapeutic response to folic acid. The diet of 2 of the animals was supplemented with 5 per cent by weight of whole dried liver substance to provide the positive controls. Observations were made for periods of forty-three to sixty-six days.

As each animal on the deficient diet developed signs of deficiency characterized by apathy, weight loss, leukopenia, muscular weakness, and diarrhea, daily subcutaneous injections of 66 micrograms of estradiol benzoate in 0.2 ml. of corn oil were made for five to ten days. In normal animals, such treatment results in highly characteristic changes in the external genitalia and the sexual skin (E. Allen, F. L. Hisaw, and W. U. Gardner, "Sex and Internal Secretions," *Ed. 2, p. 452, Chapter VIII, The Williams & Wilkins Co.* (*1939*)). These changes were observed to occur in the liver-supplemented control animals, and to some extent in 2 of the animals on the deficient ration. The changes were wholly lacking, however, in the other 6 animals of the deficient group. Vaginal smears of the nonresponsive, deficient animals also showed little change following estrogen administration, whereas the smears from the liver-supplemented animals showed the characteristic cornified cells.

It cannot be said positively that this failure of the deficient animals to respond to estrogen injection is due specifically to folic acid deficiency, since control animals receiving crystalline folic acid were not followed. From the known facts (1) that monkeys on similar rations do show excellent therapeutic responses to folic acid, and (2) that the negative response of folic acid deficient chicks to estrogen administration is specifically restored by administration of synthetic folic acid, it appears almost certain, however, that the lack of response to estrogen by the deficient monkeys is due to folic acid deficiency.

The existence of this relation in two such widely diverse forms as the chick and the monkey suggests that the phenomenon is of general physiologic significance. It may not be too early to generalize and say that all rapid proliferation of cells is dependent upon this vitamin, which appears from microbiologic evidence (L. L. Rogers and W. Shive, *J. Biol. Chem.* **172,** *751* (*1948*), J. L. Stokes, *J. Bact.* **48,** *201* (*1944*)) to be specifically involved in the synthesis of thymine and of purine bases, substances necessary for the formation of the nuclear material of all cells.

EFFECT OF THIAMINE DEFICIENCY ON WESTERN EQUINE ENCEPHALITIS IN MICE

Previous reviews (*Nutrition Reviews* **3,** *69* (*1945*)) concluded that thiamine deficiency increases the resistance of mice to the Lansing strain of poliomyelitis, at least as judged by the number of paralyzed animals and the death rate. A comparable effect has not been found in association with vitamin B-complex deficiency in the cotton rat injected with the same virus (H. M. Weaver, *Am. J. Dis. Child.* **69,** *26* (*1945*)). In avian encephalomyelitis of chicks the role of thiamine has not been

clearcut, the susceptibility of the chicks depending on a number of factors including the age of the chick, the previous state of nutrition, and the amount of thiamine fed (J. M. Cooperman, H. C. Lichstein, P. F. Clark, and C. A. Elvehjem, *J. Bact.* **52,** *467 (1946)*). In poliomyelitis of monkeys, thiamine deficiency produced no changes either in susceptibility or on the course of infection (P. F. Clark, H. A. Waisman, H. C. Lichstein, and E. S. Jones, *Proc. Soc. Exp. Biol. Med.* **58,** *42 (1945)*).

E. B. Kearney and associates (*J. Inf. Dis.* **82,** *177 (1948)*) report studies in mice in which the relation of thiamine deficiency to Western equine encephalomyelitis is described. This experiment was undertaken to determine what the effect on another neurotropic virus infection might be. The strain of virus used was mouse-adapted, and had been carried in the Wisconsin laboratory for the past five years. The source of the virus was infected mouse brain, usually preserved as a frozen 10 per cent suspension in normal saline or phosphate buffer. The study was carried out in approximately 450 animals.

When the mice were inoculated after severe signs of thiamine deficiency were evident, usually after seventeen to nineteen days on the diet, the majority failed to show characteristic signs of infection. Such mice showed only weakness progressing to atonia, tremors, and difficulty in maintaining balance or in righting themselves. The time of death was slightly delayed as compared with mice on the optimum diet in two of the five series, but was always earlier than in uninoculated deficient mice or in those inoculated with a suspension of normal brain.

Mice on the deficient diet when inoculated before the deficiency was marked (eleven days) showed characteristic signs of infection, as did those receiving 30 or 60 micrograms of thiamine per 100 g. of diet.

To determine the cause of death in these mice the last two series were checked continuously from 8:00 a.m. until 10:00 p.m. each day. Some were injected with a solution of thiamine and glucose in an attempt to distinguish deficiency signs from those of infection. At the time of death, mice were autopsied, bacteriologic studies were made of the heart blood and intestinal contents, and brains were removed for histologic study and titration of virus content. Injections of thiamine and glucose were of little aid in interpreting the signs presented by the mice for they produced in some transitory stimulation and alleviation of deficiency signs but no more definite signs of infection. Bacteriologic studies proved negative. Titration of pooled brains indicated multiplication of the virus in these deficient mice and histologic sections of the brains showed evidence of encephalomyelitis although typical signs of the disease were lacking.

It appears therefore that thiamine deficiency markedly alters the course of Western equine encephalomyelitis virus infection in mice, but shows little effect on final mortality rates. It does not prevent multiplication of the virus in the brains of mice nor the production of characteristic brain lesions. Significantly, severe restrictions in total food intake or in caloric intake when the diet was adequate did not alter the course of infection; inanition itself, therefore, cannot be responsible for the effects of thiamine deficiency.

The thought that the deficiency modified the peripheral signs of infection by depressing the muscular responses to central nervous system stimulation could not be supported by observing the effects of injection of thiamine and glucose for, following such injections, the muscular responses to central nervous system stimulation were not altered and more typical encephalitic signs did not appear.

NOTES

Letter to the Editor

Dear Sir:

I was most interested in the article on "Dental Caries Experience in Malnourished Children" (*Nutrition Reviews* **6,** *155 (1948)*).

I believe, however, that it is a pity that no reference is given to the observations made in Europe during the last war in Belgium and in France, when the same findings were made quite unexpectedly. The data are absolutely striking. This was described in F. Watry's report for Belgium and M. Dechaume's report for Paris ("VII^{e} Congrès de Chimie Biologique, Liège, 3–6 Octobre, 1946," *pp. 366 to 375, Masson, Paris (1947)*).

E. J. Bigwood
University of Brussels
Belgium

Sodium in Canned Foods

The low salt diet is among the most important therapeutic measures in the physician's armamentarium. Without rigid restriction of salt many patients with heart failure do not improve despite other therapeutic measures. Patients with hypertension, renal disease, and certain forms of liver disease often benefit greatly from diets containing less than 1 g. of sodium chloride daily.

Preparation of a low sodium diet requires great care to avoid hidden sources of sodium (for example, sodium bicarbonate and sodium benzoate). It is not generally recognized that canned foods, especially vegetables, contain more salt than those freshly cooked without salt. For example, fresh green beans contain 0.8 mg. of sodium per 100 g., while for canned green beans the value is 410 mg. per 100 g. ("Sodium and Potassium Analyses of Foods and Waters," *Fifth List, Mead Johnson and Company, October (1947)*). A few canners have prepared vegetables without added salt, but the supply is still small.

Composition of Sow's Milk

Modern studies of the composition of milk of various species which have been reviewed in this journal have dealt with goat's milk (*Nutrition Reviews* **3,** *203 (1945)*), cow's milk (*Ibid.* **3,** *118 (1945)*; **5,** *20 (1947)*), and human milk (*Ibid.* **4,** *134 (1946)*). An extensive investigation of the composition of the milk of the sow during various portions of the lactational cycle has been reported by R. Braude and co-workers (*Brit. J. Nutrition* **1,** *64 (1947)*). Twelve lactation periods of 9 sows were included. The colostrum or milk was collected by manual expression following injection of the oxytocic principle of the pituitary. The samples were analyzed for total solids, fat, vitamin A, ascorbic acid, thiamine (free and total), and riboflavin, and some of them were further analyzed for protein, lactose, ash, calcium, phosphorus, and vitamin D. The data are presented by season and by day of lactation.

The mean values for colostrum were (in grams per 100 g.) as follows: total solids, 25.16 ± 0.96; fat, 4.05 ± 0.43; nonfat solids, 21.11 ± 0.87; protein, 17.77 ± 0.93; lactose, 3.46 ± 0.09; calcium, 0.053 ± 0.005; phosphorus, 0.082 ± 0.008; vitamin A, 71.1 ± 9.1 I.U. per gram of fat; ascorbic acid, 30.6 ± 2.0 mg. per 100 ml.; total thiamine, 96.8 ± 7.8 micrograms per 100 ml.; free thiamine, 70.8 ± 7.8 micrograms per 100 ml.; riboflavin, 44.9 ± 5.7 micrograms per 100 ml. The ascorbic acid content was lower and the riboflavin content was higher in summer than in winter.

The mean values for milk from the tenth to fifty-sixth day of lactation (grams per 100 g.) were: total solids, 19.87 ± 0.14; fat, 8.17 ± 0.17; nonfat solids, 11.67 ± 0.08; protein, 5.79 ± 0.08; lactose, 4.81 ± 0.05; calcium, 0.250 ± 0.006; phosphorus, 0.166 ± 0.002; vitamin A, 11.0 ± 0.4 I.U. per gram of fat; ascorbic acid, 13.0 ± 0.3 mg. per 100 ml.; total thiamine, 67.7 ± 1.8 micrograms per 100 ml.; free thiamine, 14.4

± 1.2 micrograms per 100 ml.; riboflavin, 45.7 ± 1.7 micrograms per 100 ml. The vitamin A content was higher and the total thiamine content was lower in summer than in winter. The vitamin D content of a pooled sample of milk fat was 0.55 I.U. per gram of fat.

The authors note that the composition of sow's milk was similar to that of ewe's milk except for the high ascorbic acid content and low riboflavin value.

Colitis in the Folic Acid Deficient Monkey

J. F. Rinehart and L. D. Greenberg (*Am. J. Path.* **24,** *710 (1948)*) described the lesions of the colon in the rhesus monkey which develop concurrently with the extreme diarrhea occurring during periods of folic acid deficiency. Postmortem examinations of 7 folic acid deficient monkeys were made; of these, 6 showed a structural colitis. In the earlier phases, small irregular ulcers with purulent exudate were observed in the congested and edematous mucosa. These lesions became more extensive as the deficiency became more severe. Cystic dilatation was seen in the mucosal glands which were lined by atrophic and degenerating epithelial cells. Mucous secretory activity appeared to be reduced below normal and the nuclei of many of the secretory cells were hyperchromatic. Of particular interest was one monkey which had been maintained in a state of chronic deficiency for twenty-three months. A complete loss of the mucosa and the formation of a rigid, thick-walled colon with narrow lumen had taken place. Stool cultures were made on 6 of the folic acid deficient monkeys to determine whether a concurrent bacillary dysentery had been responsible for the lesions. The Flexner dysentery bacillus was isolated from only two of these stools indicating possibly that the ulcerative condition was not entirely dependent upon a recognizable pathogen but might be largely due directly to the deficiency disease. The lesions of the colon in both the acute and chronic phases of folic acid deficiency were described as having definite similarities to comparable phases of ulcerative colitis in man. It was suggested by these investigators that the possibility of folic acid deficiency as a factor in the pathogenesis of ulcerative colitis in man merited a critical clinical survey.

Recent Nutrition Books

The 1947 Year Book of Endocrinology, Metabolism, and Nutrition. The Year Book Publishers, Inc., 304 South Dearborn St., Chicago, Ill. 1948. Pp. 575.

Live Long and Like It. C. Ward Crampton. Public Affairs Pamphlet No. 139. (Can be obtained from: Public Affairs Committee, Inc., 22 East 38th St., New York 16, N. Y. Price: 20 cents each). 1948. Pp. 31.

FIAT Review of German Science, Biochemistry, Part I. Richard Kuhn. Price: In microfilm $6.50 for the volume. Photostat $23.00 for the volume. Orders to be addressed to the Library of Congress, Photo-duplication Service, Publication Board Project, Washington 25, D. C. Check or money order made payable to the Librarian of Congress.

Advances in Food Research. Vol. I. Edited by E. M. Mrak and G. F. Stewart. Academic Press, Inc., New York, N. Y. 1948. Pp. 459. Price: $7.50.

Fatty Liver Disease in Infants in the British West Indies. J. C. Waterlow. His Majjesty's Stationery Office, London. 1948. Pp. 84, plus XII plates. Price: two shillings. (May be obtained from British Library of Information, 50 Rockefeller Plaza, New York, N. Y.)

Thyroid Enlargement and Other Changes Related to the Mineral Content of Drinking Water (With a Note on Goitre Prophylaxis). Medical Research Council Memorandum No. 18. Margaret M. Murray, J. A. Ryle, Beatrice W. Simpson, and D. C. Wilson. His Majesty's Stationery Office, London. 1948. Price: Ninepence net.

BOARD OF TRUSTEES

*KARL T. COMPTON, *Chairman*
President, Massachusetts Institute of Technology

*CASON J. CALLAWAY, *Vice Chairman*
Hamilton, Georgia

JOSEPH F. ABBOTT, *President,*
American Sugar Refining Co.
PAUL ARMSTRONG,
General Manager,
California Fruit Growers Exchange
A. C. BENJAMIN, *Manager,*
"Junket" Brand Foods,
Chr. Hansen's Laboratories
CARLYLE H. BLACK,
Executive Vice President,
American Can Co.
*F. G. BOUDREAU, *Chairman,*
Food and Nutrition Board,
National Research Council
R. K. BRODIE, *Vice President,*
The Procter & Gamble Company
FRANK E. BUCK, *President,*
Golden State Co., Ltd.
ELLSWORTH BUNKER, *President,*
National Sugar Refining Co.
*O. C. CARMICHAEL, *President,*
The Carnegie Foundation for the Advancement of Teaching
*REV. JOHN J. CAVANAUGH,
President, Notre Dame University
*W. C. COFFEY,
President Emeritus,
University of Minnesota
CARLE C. CONWAY, *Chairman,*
Continental Can Co., Inc.
GEORGE H. COPPERS, *President,*
National Biscuit Company
E. B. COSGROVE, *President,*
Minnesota Valley Canning Co.
DANIEL W. CREEDEN,
President,
Libby, McNeill & Libby
*CHARLES WESLEY DUNN,
Member of New York Bar
A. W. EAMES, *President,*
California Packing Corp.
GEORGE A. EASTWOOD,
Chairman,
Armour and Company
EDGAR J. FORIO,
Vice President,
The Coca-Cola Company
CLARENCE FRANCIS, *Chairman,*
General Foods Corp.
*E. B. FRED, *President,*
University of Wisconsin
TALBOT O. FREEMAN,
Vice President,
Pepsi-Cola Company
FRANK GERBER, *Chairman,*
Gerber Products Co.
*RUFUS C. HARRIS, *President,*
Tulane University
HENRY J. HEINZ II, *President,*
H. J. Heinz Co.
JOHN HOLMES, *President,*
Swift & Company
*CHARLES GLEN KING, *Scientific Director of the Nutrition Foundation, Inc.*
JAMES E. KNOX,
General Manager,
Chas. B. Knox Gelatin Co., Inc.
J. PRESTON LEVIS, *President,*
Owens-Illinois Glass Co.
C. R. LINDBACK, *President,*
Abbott's Dairies, Inc.
K. D. LOOSE, *Vice President,*
Sunshine Biscuits Inc.
ARTHUR MCCALLUM, *President,*
Flako Products Corp.
CHARLES P. MCCORMICK,
President,
McCormick & Company
JAMES MCGOWAN, JR.,
President,
Campbell Soup Co.
J. S. MCLEAN, *President,*
Canada Packers, Limited
ARMAND MAY, *Chairman,*
American Lecithin Co., Inc.
JOHN T. MENZIES, *President,*
The Crosse & Blackwell Co.
JOEL MITCHELL, *President,*
Standard Brands, Inc.
WILLIAM T. NARDIN, *Vice President and General Manager,*
Pet Milk Company
VICTOR T. NORTON, *Vice President and General Manager,*
American Home Foods, Inc.
WALTER P. PAEPCKE, *Chairman,*
Container Corp. of America
*THOMAS PARRAN,
Surgeon General,
U. S. Public Health Service
RUSSELL G. PARTRIDGE, *Representing United Fruit Company*
D. B. PECK, *President,*
Bowman Dairy Company
MARIO PERUZZI, *President,*
Planters Nut & Chocolate Co.
PHILIP W. PILLSBURY,
President,
Pillsbury Mills, Inc.
R. A. ROBERTSON,
Vice President,
George Weston, Limited
MORRIS SAYRE, *President,*
Corn Products Refining Co.
OTTO SCHNERING, *President,*
Curtiss Candy Company
*GEORGE A. SLOAN, *President of the Nutrition Foundation, Inc.*
B. E. SNYDER,
President and Treasurer,
R. B. Davis Company
NORMAN W. STEWART,
President,
E. Pritchard, Inc.
VERNON STOUFFER, *President,*
The Stouffer Corporation
R. DOUGLAS STUART,
Vice-Chairman,
The Quaker Oats Co.
G. CULLEN THOMAS,
Vice President,
General Mills, Inc.
L. A. VAN BOMEL, *President,*
National Dairy Products Corp.
W. H. VANDERPLOEG, *President,*
The Kellogg Company
J. V. VOSBURGH, *Vice President,*
Beech-Nut Packing Company
L. A. WARREN, *President,*
Safeway Stores, Inc.
*RAY LYMAN WILBUR,
Chancellor,
Stanford University
*M. L. WILSON, *Chief,*
Nutrition Programs,
U. S. Department of Agriculture
CHARLES L. WIRTH, *President,*
Red Star Yeast and Products Co.
*STEPHEN S. WISE, *Rabbi,*
Free Synagogue
C. I. WOOD, *Vice President,*
Thomas J. Lipton, Inc.
HARRY W. ZINSMASTER,
President,
Zinsmaster Baking Co.

OLE SALTHE, *Secretary*

---

* Asterisk (*) indicates Trustees as representative of the public.

THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress, and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

Signed articles do not necessarily represent the opinion of the Editorial Staff.

A leaflet based on articles appearing currently in NUTRITION REVIEWS and entitled "Current Research in the Science of Nutrition" is distributed free of charge by the Nutrition Foundation each month. The vocabulary of the leaflet is adapted for lay reading and the subject matter is restricted to clearly established findings of practical interest. Those who wish to have their names placed on the mailing list should write directly to the Nutrition Foundation, Inc., Chrysler Building, New York City.